Universität Rostock Traditio et Innovatio

Encrustation of charophyte species – seasonality and habitat specificity

Saisonale und Habitat-spezifische Karbonatausfällung von Characeen Arten

Dissertation
zur
Erlangung des akademischen Grades
doctor rerum naturalium (Dr. rer. nat.)
der Mathematisch-Naturwissenschaftlichen Fakultät
der Universität Rostock

vorgelegt von Anne Herbst,
geb. am 24.10.1988 in Braunschweig
aus Rostock

Rostock, 29.06.2018

urn:nbn:de:gbv:28-diss2018-0155-8

Gutachter:

1. Prof. Dr. Hendrik Schubert, Universität Rostock, Institut für Biowissenschaften, Aquatische Ökologie
2. Prof. Dr. Mariusz Pełechaty, Adam Mickiewicz University, Department of Hydrobiology

Datum der Einreichung: 29.06.2018
Datum der Verteidigung: 19.10.2018

Contents

Zusammenfassung

Karbonatfällung und Elementzusammensetzung verschiedener Characeenarten wurden mit dem Ziel untersucht, den Mechanismus der Präzipitation aufzuklären. Die hier vorgestellten Untersuchungen konzentrierten sich auf die Erfassung der 1) Saisonalität, 2) Artspezifität und 3) Habitatspezifität der Karbonatfällung. Dazu wurden Characeen aus verschiedenen Habitaten (Süßwasser, marinem Brackwasser und Binnensalzstandorten) analysiert sowie Laborinkubationen durchgeführt.

Der Einfluss der Saisonalität wurde an sechs Characeenarten aus zwei Hartwasser Seen untersucht; die Probenahme erfolgte monatlich über den Zeitraum eines Jahres. Eine ausgeprägte Saisonalität der Kalkfällung konnte für *Chara contraria*, *C. subspinosa* und *Nitellopsis obtusa* im Krüselinsee sowie für *C. globularis* und *C. tomentosa* im Lützlower See nachgewiesen werden. Der Befund, dass *C. subspinosa* im Lützlower See und *C. tomentosa* im Krüselinsee keine Anzeichen einer Saisonalität der Kalkfällung zeigten, weist auf einen starken Einfluss von Habitatparametern auf die Ausprägung der Saisonalität hin. Die Saisonalität der Karbonatausfällungen zeigten Art-spezifische Muster auf; es wurden negative Korrelationen mit der Konzentration des gesamten anorganischen Kohlenstoffes nachgewiesen.

Ein Vergleich der Karbonatfällung und Elementzusammensetzung zwischen Individuen von Süß- und Brackwasserstandorten ergab signifikante Unterschiede in der Elementzusammensetzung und Ausmaß der Karbonatfällung. Der Karbonatgehalt je Trockengewicht war bei Individuen, die in Süßwasser wuchsen, höher verglichen mit Brackwasserstandorten. Die Artspezifität der Unterschiede deutet auf einen starken Einfluss physiologischer Mechanismen hin, der Einfluss physikalisch-chemischer Eigenschaften des Wassers auf die Ausprägung der Kalkkrustationen ist ein weiterer Faktor, der diese Unterschiede Art-unabhängig beeinflusst. Das zeigte sich vor allem bei den Elementanalysen, die im Fall von Ca und Mg Habitatspezifität aufwiesen, während für K eine Artabhängigkeit gefunden wurde. Für Na wurde sowohl eine Art- als auch eine Habitatabhängigkeit nachgewiesen; für P wurde kein spezifisches Muster gefunden.

Die Ergebnisse der Analysen von Individuen aus Binnensalzstandorten mit starken Ionenanomalien bestätigen diese Ergebnisse, auch hier wurde für Na eine ausgeprägte Habitatspezifität nachgewiesen. Bei Inkubationen in Schwermetall-belastetem Wasser konnte eine Co-Präzipitation von Cd, Cu und Zn in der Karbonat Kruste nachgewiesen werden. Pb dagegen konnte, obwohl im verwendeten Wasser vorhanden, nicht in den Krusten nachgewiesen werden. Es wird vermutet, dass Pb im Sediment ausfällt.

Die Hypothesen wurden somit bestätigt; es wurde nachgewiesen, dass sowohl Saisonalität als auch Habitat-spezifische Wasserparameter die Karbonatfällung von Characeen in Artspezifischer Weise beeinflussen. Darüber hinaus konnte die prinzipielle Einsatzbarkeit von Characeen zur Bioremediation von Abwässern aus dem Kupferbergbau nachgewiesen werden.

1 Summary

Encrustation and element content of different charophytes were studied in order to identify the main factors decisive for extracellular carbonate precipitation. For testing the hypotheses that precipitation is 1) dependent from seasonality, 2) species-specific and, 3) influenced by water chemistry parameters, charophyte species growing in different habitats (freshwater, marine-brackish, and inland brackish water sites with strong ion anomalies) were studied.

For resolving seasonality of encrustation, six charophyte species from two hard-water lakes were investigated monthly for a period of one year. Seasonal patterns were analysed for the interaction with water chemistry. Encrustation followed a seasonal pattern for *Chara contraria*, *C. subspinosa* and *Nitellopsis obtusa* in Krüselinsee and for *C. globularis* and *C. tomentosa* in Lützlower See. However, no seasonality in the precipitated $CaCO_3$ was observed for *C. subspinosa* in Lützlower See and for *C. tomentosa* in Krüselinsee, indicating a habitat-specific dependency. Seasonal patterns of charophyte encrustation were species-specific and correlated negatively with the concentration of total inorganic carbon (TIC) of both lakes. Seasonality and TIC were the most important parameters determining the element composition of investigated charophytes.

A comparison of encrustation and element content across species growing in freshwater and brackish water was conducted. Significant differences in encrustation of charophytes were found between FW and BW sites. Individuals from FW had a far higher carbonate content based on dry weight than individuals from BW. In BW, *C. tomentosa* was less encrusted than *C. aspera*. The effect observed was caused by physico-chemical characteristics and physiological mechanisms, the latter being species-specific and consequently leading to species-specific differences in encrustation of *C. aspera* and *C. tomentosa* under BW conditions. The K/Na-ratio differed between FW and BW in a species-specific manner. Element composition was habitat-specific for Ca and Mg, species-specific for K, and habitat- and species-specific for Na. P contents showed no specific pattern.

In brackish waters with a strong ion anomaly, ion composition rather than ion concentration, and especially the Na^+ ratio to other ions, is decisive for the encrustation of charophytes. The carbonate composition of charophytes (Ca > K > Mg > Na > P > Mn) was shifted; the position of Na changed according to Na^+ concentration of the habitat water. Charophytes withstand the heavy metal concentrations of Schlüsselstollen water when diluted. Cd, Cu and Zn were co-precipitated in the carbonate crust of charophytes. Pb was not detected in the carbonate crust, probably having been deposited in the sediments. Charophytes can be used for bioremediation of diluted Schlüsselstollen water by co-precipitation of Cd, Cu and Zn.

Both seasonality, as well as site-specific water chemistry parameters, impact on the encrustation of charophytes. For seasonality and site-specificity, pronounced species-specific patterns were observed which confirms the investigated hypotheses.

2 Introduction

Charophytes are macrophytes of the order Charales Lindley 1836 belonging to the Streptophyta (Figure 1) (Jeffrey 1967; Stewart and Mattox 1975). This lineage also includes the Embryophyta, thus charophytes are closely related to land plants (Jeffrey 1967; McCourt 1995). Charophytes exhibit a complex morphology and reproduce by sexual as well as vegetative mechanisms (Figure 2) (Fritsch 1935; Krause 1997; Graham et al. 2000).

Charophytes can form dense meadows which provide habitats and shelter for epiphytes, invertebrates and fish, indicating their important ecological function in the ecosystem (Kairesalo et al. 1987; Kingsford and Porter 1994; Kuczyńska-Kippen 2007). Furthermore, characean stands stabilise the sediment, reducing sediment resuspension and turbidity and thereby enhancing the water transparency (Scheffer et al. 1993; Van den Berg et al. 1998a; Blindow et al. 2002). These mechanisms may stabilise the clear-water state in shallow lakes (Van den Berg et al. 1998b; Scheffer 2001) which include a complex pattern of trophic interactions, initiated by their habitat-engineering function (Hargeby et al. 1994; Schubert et al. 2018a).

Domain:	Eukaryota
Kingdom:	Plantae
Phylum:	Viridiplantae
	Streptophyta
Division:	Charophyta
Class:	Charophyceae
Order:	Charales
Family:	Characeae
Genera:	*Chara*
	Lychnothamnus
	Lamprothamnium
	Nitella
	Tolypella
Family:	Feistiellaceae
Genus:	*Nitellopsis*

Figure 1: Systematics of charophytes. Data from Guiry and Guiry (2018).

Figure 2: Morphology of charophytes. Thallus of *C. vulgaris*, scale bar = 5 cm (A), gametangia of monoecious *C. subspinosa* (B), and oogonia of female (C) and antheridia of male (D) *C. tomentosa* (dioecious), scale bar = 1 mm.

The presence or absence of charophytes seems to depend on abiotic lake characteristics; especially water chemistry plays an important role (Van den Berg et al. 1998a; Blindow et al. 2014). They often occur in waters with low nutrient concentrations. Thus, some charophytes can serve as bioindicators for nutrient-poor conditions (Krause 1981; Melzer 1994; Doege et al. 2016). In clear-water ecosystems charophytes are more competitive than other macrophytes but are outcompeted under eutrophic conditions (Ozimek and Kowalczewski 1984; Pieczyńska et al. 1988; Blindow 1992a). However, charophytes are not limited to oligotrophic waters, also inhabiting eutrophic waters when niches are available (Figure 3) (Kufel and Kufel 2002; Schubert et al. 2018a). Being pioneers, characean plants are often the first to colonise ephemeral waterbodies (Casanova and Brock 1990; Blindow 1992b; Van den Berg et al. 1998b; Bicudo and Bueno 2013; Schubert et al. 2018a).

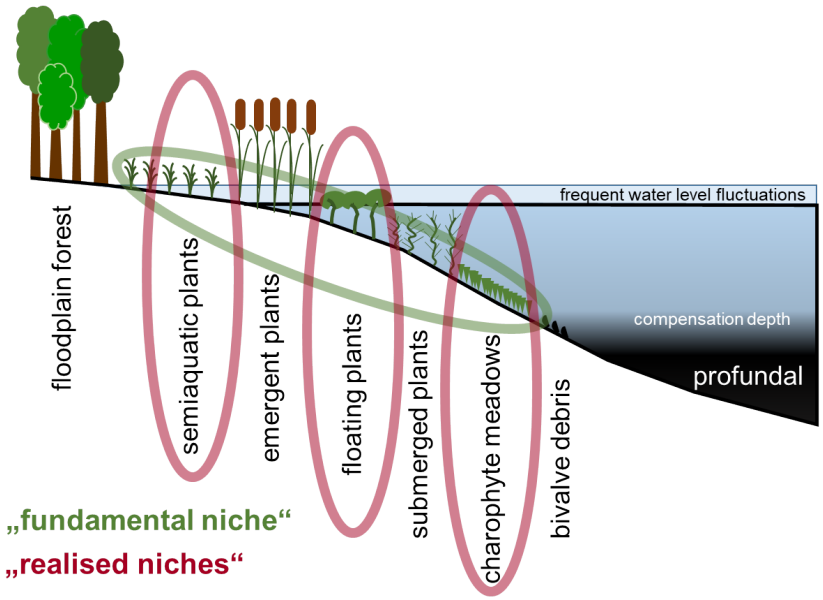

Figure 3: Fundamental and realised niches of charophytes. Drawing: Schubert et al. (2018a).

In general, charophytes grow in fresh, brackish and saline waters (Krause 1997; Blindow 2000). Most species occur in freshwater and only a few are able to withstand, or are restricted to, brackish or saline conditions (Schubert and Blindow 2003). Examples for these groups are: *C. subspinosa* Rupr. 1846 and *Nitellopsis obtusa* Groves 1919 as freshwater species, *C. aspera* Willd. 1809 and *C. tomentosa* L. 1753 can be found under freshwater as well as brackish water conditions (Hasslow 1931; Wahlstedt 1862). *C. canescens* Loisel. 1810 is restricted to brackish water only and *Lamprothamnium papulosum* Groves 1916 inhabiting brackish and saline waters (Wood and Imahori 1965; Krause 1997).

Charophytes are distributed along a wide pH range (Doege et al. 2016; Schubert et al. 2018b). Most species of the genus *Nitella* occur in acid to neutral waters, whereas most *Chara*-species are most frequently found at alkaline hard-waters (Blindow and van de Weyer 2016). An exception is *C. braunii* Gmelin 1826, an ecorticated species (Figure 4), that grows mainly in soft waters (Krause 1997; Doege et al. 2016; Schubert et al. 2018b).

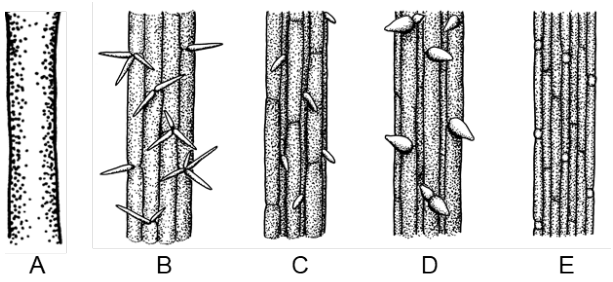

Figure 4: Schematic drawing of cortical structures in different charophytes. Ecorticated *C. braunii* (A) and cortication of haplostichous *C. canescens* (B), diplostichous, aulacanthous *C. vulgaris* (C), diplostichous tylacanthous *C. tomentosa* (D), triplostichous *C. globularis* (E). Drawings: Schubert and Blindow (2003).

In hard-water lakes, charophytes encrust on the plant surfaces due to alkalinisation of the surrounding water in the process of photosynthetic uptake of dissolved inorganic carbon (Smith 1967; Lucas et al. 1983; Pentecost 1984; McConnaughey 1991). Bicarbonate (HCO_3^-), the main carbon source in alkaline hard-water lakes (Figure 5) can be utilised by most species of the genus *Chara* (Wetzel 1975; Van den Berg et al. 2002; Sand-Jensen et al. 2018). It has been shown that bicarbonate was used more efficiently by *C. aspera* compared to *Stuckenia pectinata* (L) Börner 1912 for photosynthesis (Van den Berg et al. 2002). Therefore, it is concluded that charophytes have a competitive advantage over other macrophytes in hard-water lakes.

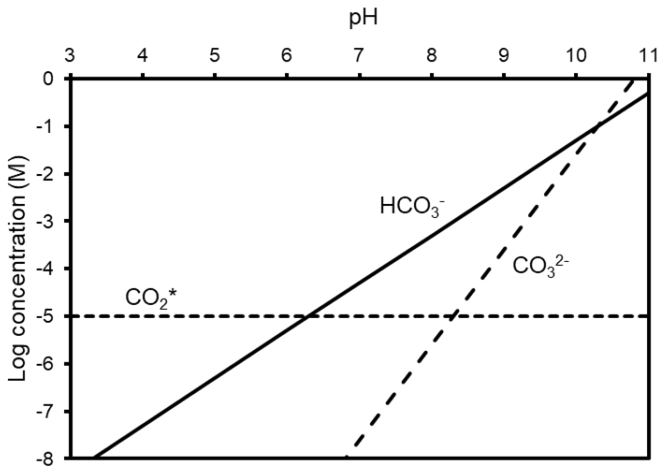

Figure 5: pH dependency of the concentrations of the dissolved inorganic carbon species at instantaneous equilibration with atmospheric CO_2 (400 µatm) by gas exchange. Drawing: Schubert et al. (2017).

The carbon uptake mechanisms in charophytes have been studied by several authors (Lucas and Smith 1973; Lucas 1975; Smith and Walker 1980; Mimura and Shimmen 1994; Bulychev et al. 2001; Ray et al. 2003). Beilby and Bisson (2012) summarised two possible uptake mechanisms (Figure 6A). Active transport of H^+ acidifies the plant surface, which changes

the concentration of dissolved inorganic carbon (DIC) species. Carbon can be taken up as H_2CO_3 and CO_2 by diffusion or via H^+:HCO_3^- symporter into the cytoplasm (Beilby and Bisson 2012). The carbonic anhydrase catalyses the conversion into CO_2, which enters the Calvin cycle in the chloroplast (Smith 1968; Lucas 1975). The remaining hydroxyls (OH^-), conversion of HCO_3^- into CO_2, are transported in the cytoplasmic streaming and are released forming exterior alkaline zones (Beilby and Bisson 2012 and references therein). In these alkaline zones, Ca^{2+} and CO_3^{2-} are precipitated as $CaCO_3$ on the plant surface (Smith and Walker 1980; McConnaughey and Falk 1991; McConnaughey and Whelan 1997).

Calcium carbonate can also be precipitated as a side-effect of active ion transport for nutrient uptake (McConnaughey and Whelan 1997; Ullrich et al. 1998; Brownlee and Taylor 2002). In both cases, local H^+ extrusion causes bicarbonate and nutrient uptake in plants (Santi et al. 1995; McConnaughey and Whelan 1997). On a microscale, at least in ecorticated charophytes, a regular pattern of acidified and alkalinised zones becomes visible as banding pattern along the internodes (Figure 6B), whereas the banding is less pronounced in corticated species (Figure 6C) (Migula 1897; Spear et al. 1969; McConnaughey and Falk 1991; Ray et al. 2003; Kawahata et al. 2013).

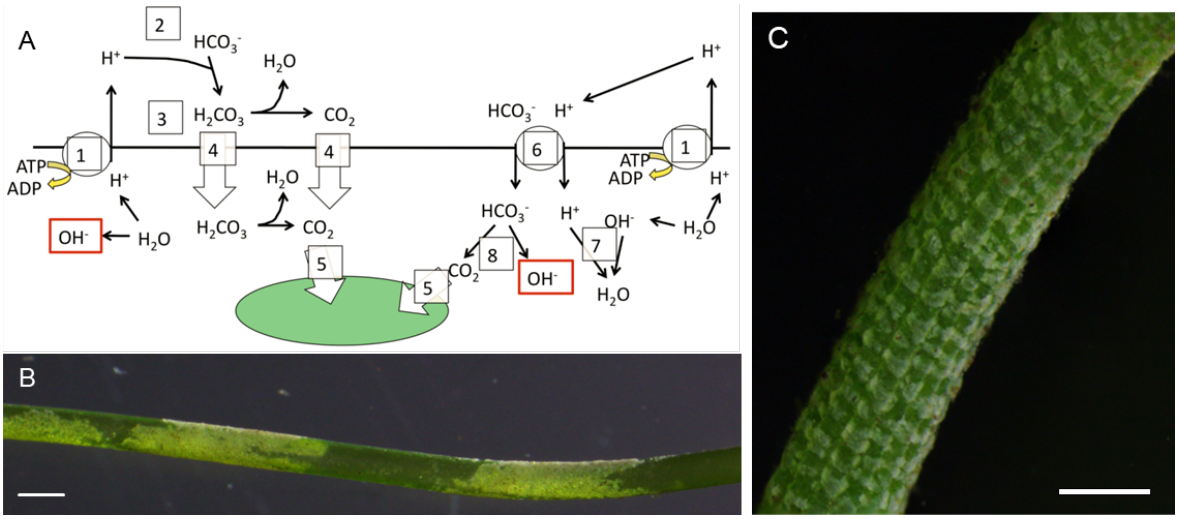

Figure 6: A. Uptake of dissolved inorganic carbon in the acid zone of charophytes (schematic diagram: Beilby and Bisson 2012). Active H^+ transport (1) acidified the plant surface, protonating HCO_3^- (2, 3). H_2CO_3, CO_2 diffuse (4) and HCO_3^- is taken up via H^+:HCO_3^- symporter (6) into the cytoplasm. H^+ neutralise OH^- (7). Carbonic anhydrase convert HCO_3^- into CO_2 (8) which enters the Calvin cycle in the chloroplast (5). B. Internode of ecorticated *C. braunii*. C. Internode of corticated *C. vulgaris*, scale bar= 500 µm.

Encrustation of charophytes is more effective in comparison to vascular plants (van den Berg et al. 2002) and can account for up to 80 % calcium carbonate of plant dry weight (Pukacz et al. 2016a). In the case of heavy encrustation of charophytes, it has been described that supersaturation with calcium carbonate of water was not required (Nõges et al. 2003; Kufel et al. 2016). Several authors pointed out the importance of site-specificity, as depth, temperature, pH, and water chemistry parameters, on the encrustation of charophytes (Kufel et al. 2013,

2016; Pukacz et al. 2016a,b). Correlation between encrustation and habitat characteristics was found to be species-specific for *C. tomentosa* and C. *globularis* Thuill. 1799 (Pukacz et al. 2016b). This could further explain species-specific differences in encrustation which was found by Kufel et al. (2013, 2016) and Pukacz et al. (2016b).

Furthermore, with encrustation being a process related to growth in general, it should exhibit a pronounced seasonality as demonstrated by, for instance, Fernández-Aláez et al. (2002). Later studies added to the knowledge of seasonality precipitate formation in charophytes (Pukacz et al. 2014b, 2016a). Seasonal pattern of charophyte encrustation found in Lake Jasne showed; precipitated carbonate contents were highest in July and August (Pukacz et al. 2014b, 2016a). These results showed that seasonality must be taken into account when comparing the relationship between water chemistry and encrustation or biomass element composition of different charophytes.

Heavy encrustations have been described for charophytes growing in freshwater lakes, but there is less encrustation in brackish water conditions. Wahlstedt (1875) already reported that brackish water charophytes (*C. aspera* and *C. tomentosa*) are far less encrusted than individuals from freshwater. Hasslow (1931) even distinguished between *C. aspera* "f. *incrustata*" from freshwater and "f. *munda*" from brackish water. In the brackish environment, charophytes have to cope with higher ion concentrations of the surrounding water, requiring osmoregulation as a countermeasure to prevent a loss of turgor (Bisson and Kirst 1995). In the process of osmotic adjustment, concentrations of Cl^-, K^+, and Na^+ are actively regulated (Winter and Kirst 1992). Specifically, the K^+/Na^+-ratio needs to be regulated, because too low ratios ($K^+/Na^+ < 1$) reduce the vitality of charophytes (Winter and Kirst 1990; Winter et al. 1996). Both, ion contents as well as turgor regulation mechanisms have been intensively studied in the laboratory and are found to differ widely among species:

Pure freshwater species (e. g. *C. corallina* Klein ex Willd. 1805 and *Nitella* spp.) keep their osmotic pressure constant by a K^+-regulation system but do not adjust their turgor under salinity changes (Bisson and Bartholomew 1984; Bisson and Kirst 1995). Oligohaline species (e. g. *C. tomentosa* and *N. obtusa*) regulate their turgor by Na^+, Cl^-, and osmolytes (sucrose), but keep K^+ concentrations constant (Winter and Kirst 1990, 1991b; Winter et al. 1999). Consequently, their K^+/Na^+-ratios drop with increasing salinity, which is why they are limited to salinities of 5-7. Mesohaline species (e. g. *C. aspera* and *C. canescens*) keep K^+ concentrations constant at low salinities and adjust turgor via Na^+, K^+, and Cl^- concentrations at higher salinities (Winter and Kirst 1991a, 1992). Mesohaline species have a reduced turgor regulation system than euryhaline species (e. g. *L. papulosum* and *C. buckellii* Allen 1951). At salinities of 20-40, euryhaline species keep Na^+ concentrations constant to obtain a high K^+/Na^+-ratio, thus tolerate the occurrence at high salinities (Hoffmann and Bisson 1990; Beilby et al. 1999).

Together with the carbonate precipitates formed on the charophyte surface, co-precipitation of phosphate and heavy metals was described (Otsuki and Wetzel 1972; Hutchinson 1975; Murphy et al. 1983; McConnaughey 1991). Interaction of heavy metals with the carbonate structure was shown by Gomes and Asaeda (2009, 2013). Encrustation of charophytes decreased in the presence of heavy metals compared to non-exposed samples (Gomes and Asaeda 2013). However, heavy metal toxicity was reduced under high calcium concentration, causing detoxification through precipitation (Gomes and Asaeda 2009; Bibi et al. 2010; Sooksawat et al. 2013, 2016).

Exposure to heavy metals affects not only growth (Taylor et al. 2000; Clabeaux et al. 2013) but also cell wall composition (Heumann 1987), formation of gametangia (Gosek et al. 1996; Rybak et al. 2017), and development of chloroplasts (Heumann 1987; Sooksawat et al. 2013) of charophytes. Heavy metals induce oxidative stress in plants resulting in enhanced generation of reactive oxygen species (ROS) (Schützendübel and Polle 2002; Asada 2006). Gao and Yan (2012) showed elevated ROS levels in *C. globularis* when exposed to Pb. Antioxidant enzymes of the defence system are inhibited at high lead concentration (≥ 40 mg L^{-1}). In an exposure experiment to Cs, growth and oxidative stress of *Nitella pseudoflabellata* Braun 1882 were measured (Atapaththu et al. 2016). Plant growth decreased and the activity of antioxidant enzymes increased during the exposure (0.1 mg L^{-1}). However, ROS levels were not enhanced significantly, which indicated the activation of defence mechanisms against oxidative stress.

Carbonate content of sediments in the littoral zone was studied by Pełechaty et al. (2013). The littoral zone, which was dominated by characean plants, measured over 80 % calcium carbonate content in the sediments (Pełechaty et al. 2013). Thus, precipitated carbonates contribute to lacustrine sedimentations which are also used in paleolimnology (Müller et al. 1972; García 1994; Pentecost et al. 2006; Apolinarska et al. 2011). For the co-precipitation, it means that heavy metals are stored in the sediments (Triboit et al. 2010; Gomes and Asaeda 2013). Gomes and Asaeda (2013) distinguished between exchangeable, organic-bound, and carbonate-bound heavy metal compounds. The highest proportion was of redox insensitive carbonate-bound compounds which ensure a long-term storage in sediments (Gomes and Asaeda 2013).

As a consequence charophytes have the potential for bioremediation by means of co-precipitation of heavy metals from the water column or by uptake of bioavailable compounds from the sediment (Figure 7) (Lacerda et al. 1992; Kalin et al. 2005; Marquardt and Schubert 2009; Schneider and Nizzetto 2012; Clabeaux et al. 2013).

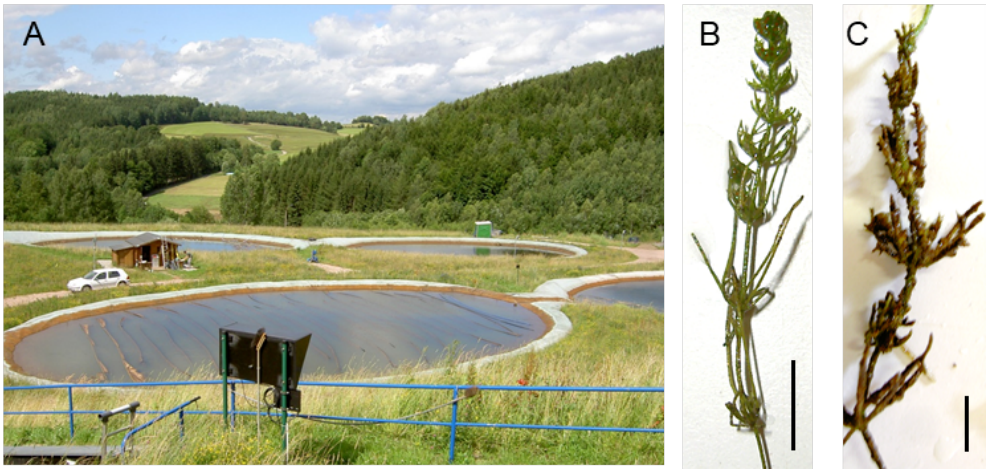

Figure 7: A. Bioremediation ponds in Pöhla (Wismut GmbH), Germany, B. Non encrusted *C. vulgaris* from the unpolluted reference site aside the bioremediation ponds, C. Heavily encrusted *C. vulgaris* from the bioremediation pond, scale bar = 1cm. Photographs: H. Schubert.

Former mining activities increase the inputs of metals and thus heightens pollution of water systems. In Germany examples for mining pollution are; former uranium mining in Thuringia and Saxony, lignite mining activities in the Lausitzer region, and the Mansfelder region where copper and potash were mined (Figure 8) (Schreck et al. 2004; Baborowski et al. 2005; Baborowski and Bozau 2006; Friese et al. 1998). Restoration measures were outlined by the Wismut GmbH for the former mining district in Thuringia and Saxony of uranium and by the RWE AG for lignite mining legacies. Nevertheless, treatments for copper mining in the Mansfelder region, because of early decommission and missing assignment of responsibility, are unsolved. The topical problem of pollution is described more precisely in the following:

Copper was mined in the Mansfelder region for more than 800 years (Hartwig et al. 1999). Despite decommission in 1969, dewatering systems are still releasing extreme saline waters to the surface (Schreck et al. 2004; FGG ELBE 2015). High concentrations of lead (3 t), copper (2.4 t), cadmium (0.5 t) and zinc (150 t) per year are leaking from the adit Schlüsselstollen and are carried into the river Schlenze, where concentrations of cadmium and lead exceed the norm by a hundredfold (FGG ELBE Anlage 7 2015; LAF/PLEJADES 2013). The Schlenze flows into the river Saale and ultimately ends in the Elbe, where the heavy metals can be detected downstream until Hamburg at least (Schreck et al. 2004). The increased heavy metal concentrations of the Schlüsselstollen water exert a lasting influence on the ecosystem; sediments and biofilms accumulate with Cu, Pb, and Zn (Mages et al. 2006; Baborowski and Bozau 2006; Baborowski and von Tümpling 2012). Consequently, action plans were established but no measures are present for substantial reducing of heavy metal concentrations from the Schlüsselstollen water (Claus et al. 2015; FGG ELBE 2015).

Figure 8: Mining residuals; Asche in front of a potash mining heap in Teutschenthal (A) and Schlüsselstollen outlet near Friedeburg (B).

In this context, bioremediation as a cost-effective process becomes of considerable interest for the removal of pollutants from the environment by means of biological activity (Oswald 1988; Olguín 2003). Bioremediation needs to be tested for application by means of laboratory and field investigations to fully employ the potential of charophytes. More details about physiological limits of species and their toxicity limits need to be available. The present study absorbed the knowledge required for a bioremediation concept.

As a result, encrustation of different charophytes was studied in habitats which are distinct with respect to water chemistry. Seasonal impact on the carbonate precipitation was also analysed which allows the investigation of the following hypotheses:

1. Encrustation of charophytes exhibit a seasonal pattern.
2. Both, seasonal pattern as well as extent of precipitation are species-specific.
3. Habitat-specificity influences on the encrustation of charophytes. Water chemistry parameters impact on the carbonate and element contents of charophytes.

3 Material and Methods

3.1 Sampling sites

The sampling sites were chosen by their charophyte flora allowing for occurrence of the *Chara hispida*-species group *sensu* Wood (1962). Investigated sampling sites and species sampled are shown in Figure 9 and Table 1.

For a period of one year two hard-water lakes, Krüselinsee (Mecklenburg Lake District) and Lützlower See (Uckermark), both located in the north-eastern part of Germany were investigated. Samples were taken monthly from April 2016 to March 2017 with exception of January due to ice cover (4.1 Seasonality and 4.2 Fertility).

In cooperation with the University of Greifswald (Germany) ten freshwater lakes and six brackish water lagoons in northern Europe were sampled by Levke Henningsen and Irmgard Blindow in June and July 2015 (4.4 Freshwater and brackish water habitats). Five of the freshwater lakes (Sabinensee, Gottsee, Kölpinsee, Parsteiner See, and Krüselinsee) were localised in the north-eastern part of Germany and the other five (Krankesjön, Börringesjön, Lyngsjön, Räpplinge, and Greby) in southern Sweden. All the brackish water lagoons were localised along the south-eastern coast of Sweden (Edenryd, Sibbaboda, Klumpudden, Loftahammar, Gällerskullaviken, and Hålviksfjärden).

In July 2016 three waters in Thuringia and Saxony-Anhalt, Germany were sampled. These included two residual mining holes: Angersdorfer Teiche, a gips pit filled with rain and groundwater and Asche in Teutschenthal, which is located close to a potash mining heap, in the lake area Mansfelder region. Bruchwiesen, a natural karstic spring, in Bad Tennstadt was also sampled (4.3 Age gradient).
In the Mansfelder region in Saxony-Anhalt, four sampling sites were investigated in August 2017 (4.5 Heavy metal exposure). Water and sediment samples were taken in the adit Schlüsselstollen before it flows into the river Schlenze, in the river Saale above and below the Schlenze discharges into the Saale, and in the Altarm of the river Saale (Figure 9).

3.2 Sample processing

Plant material was collected by snorkeling or by means of a fork directly from the shore. Undamaged individuals were picked from the gathered material and epibionts were gently removed with a brush. Charophytes were identified to the species level (Krause 1997). N value given in figures and tables represent the number of replicates analysed. For further analyses, plants were prepared in the laboratory according to the following.

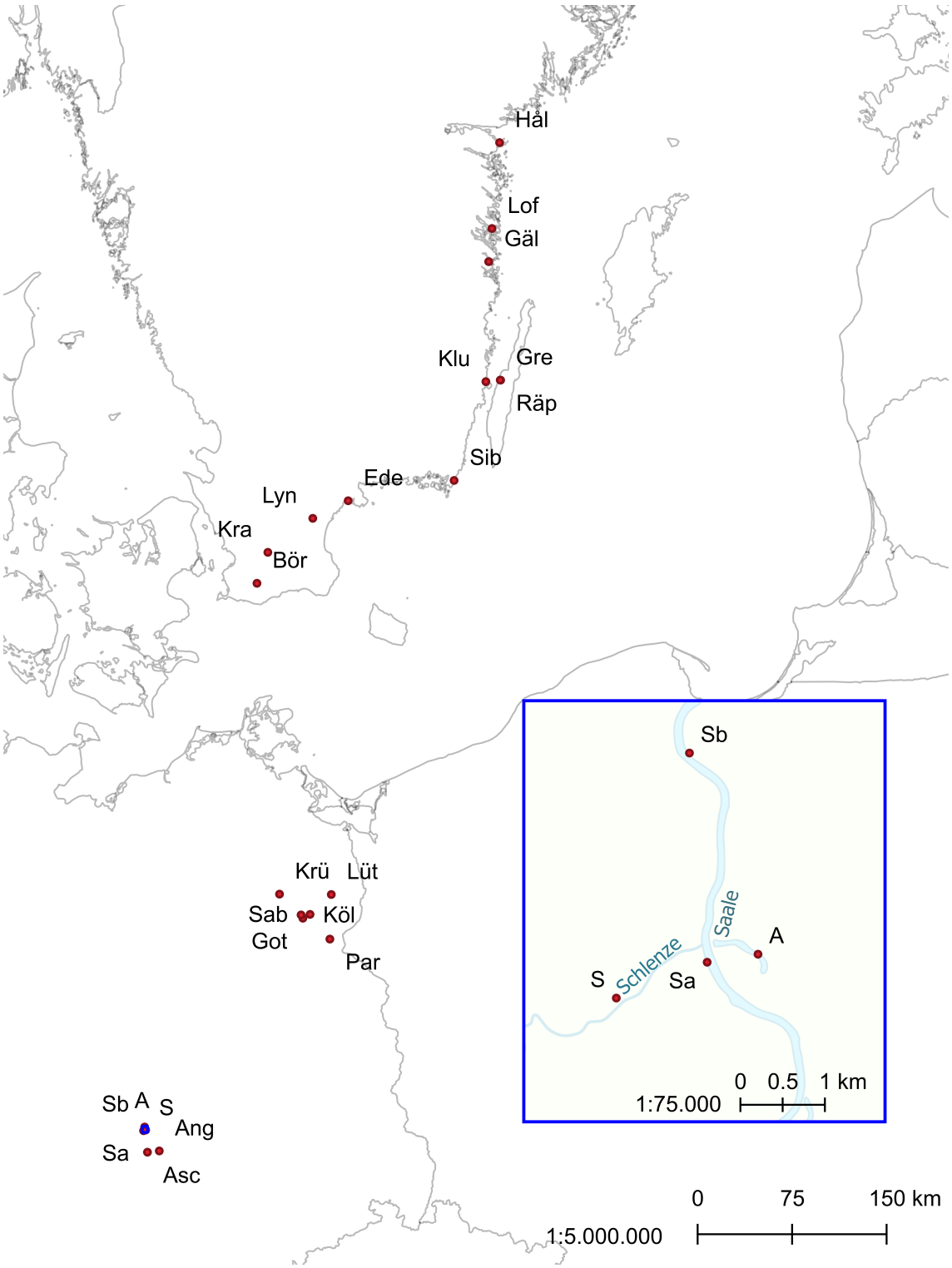

Figure 9: Map of investigated sampling sites. Sites are abbreviated as first three letters: Krüselinsee (Krü), Lützlower See (Lüt), Angersdorfer Teiche (Ang), Asche (Asc), Bruchwiesen (Bru), Sabinensee (Sab), Gottssee (Got), Kölpinsee (Köl), Parsteiner See (Par), Krankesjön (Kra), Börringesjön (Bör), Lyngsjön (Lyn), Räpplinge (Räp), Greby (Gre), Gällerskullaviken (Gäl), Hålviksfjärden (Hål), Loftahammar (Lof), Klumpudden (Klu), Edenryd (Ede), Sibbaboda (Sib), Schlüsselstollen (S), $\text{Saale}_{\text{above}}$ (Sa), $\text{Saale}_{\text{below}}$ (Sb), and Altarm (A). Sampling sites in Schlenze and Saale are shown in detail (blue box). Maps were constructed with QGIS version 3.2; data was used from GeoBasis-DE/BKG 2018 and Geoportal of the European Commission - EUROSTAT (22.12.2017).

Table 1: Investigated sampling sites with coordinates and species sampled. Species are abbreviated as first three letters: *C. aspera* (asp), *C. canescens* (can), *C. contrari*a (con), *C. globularis* (glo), *C. hispida* (his), *C. subspinosa* (sub), *C. tomentosa* (tom), *N. flexilis/opaca* (fle/opa), and *Nitellopsis obtusa* (obt).

site	abbreviation	coordinates	species
Krüselinsee	Krü	53°16'02.3" N 13°24'59.7" E	con, sub, tom, obt
Lützlower See	Lüt	53°14'44.7" N 14°01'52.2" E	glo, sub, tom, fle/opa
Angersdorfer Teiche	Ang	51°28'04.5" N 11°54'24.6" E	can, his
Asche	Asc	51°27'40.0" N 11°46'18.4" E	tom
Bruchwiesen	Bru	51°93'25.3" N 10°49'36.0" E	his
Sabinensee	Sab	53°06'44.0" N 13°45'59.4" E	sub, tom
Gottssee	Got	53°05'15.6" N 13°40'45.1" E	tom
Kölpinsee	Köl	53°06'42.1" N 13°39'35.6" E	tom, obt
Parsteiner See	Par	52°55'43.9" N 13°59'06.1" E	tom
Krankesjön	Kra	55°42'22.8" N 13°28'38.3" E	asp, sub, tom
Börringesjön	Bör	55°29'22.1" N 13°19'17.5" E	asp
Lyngsjön	Lyn	55°55'54.3" N 14°04'05.0" E	tom
Räpplinge	Räp	56°48'54.9" N 16°36'29.0" E	asp
Greby	Gre	56°48'57.3" N 16°36'11.1" E	asp
Gällerskullaviken	Gäl	57°39'52.4" N 16°36'02.3" E	tom
Hålviksfjärden	Hål	58°30'08.2" N 16°54'13.9" E	asp, tom
Loftahammar	Lof	57°53'51.8" N 16°41'13.4" E	asp
Klumpudden	Klu	56°48'51.1" N 16°24'56.1" E	tom
Edenryd	Ede	56°02'30.7" N 14°31'49.8" E	asp
Sibbaboda	Sib	56°07'53.4" N 15°53'44.4" E	asp
Schlüsselstollen	S	51°36'54.5" N 11°43'58.8" E	
$\text{Saale}_{\text{above}}$	Sa	51°37'07.4" N 11°44'55.2" E	
$\text{Saale}_{\text{below}}$	Sb	51°38'27.7" N 11°44'47.4" E	
Altarm	A	51°37'10.0" N 11°45'26.6" E	

3.2.1 Plant samples

Loss of ignition

Plants were analysed for the carbonate proportion of dry weight (DW). Carbonate content was determined by the loss of ignition (LOI) method as described by Heiri et al. (2001). The samples were dried at 60 °C (UM 400, Memmert) overnight. Plant DW was weight and combusted in crucibles by a two-step process at 550 °C and 925 °C for two hours, respectively (LE 6/11/B 150, Nabertherm). The weight loss at 550 °C represents the ash free dry weight (AFDW), while at 925 °C carbonate bounded CO_2 evolved (Equations 1 and 2). Carbonate content was calculated after Pełechaty et al. (2013) by multiplying CO_2 loss by 1.36 (fraction of CO_2 in ${CO_3}^{2-}$) to obtain ${CO_3}^{2-}$.

$$\text{AFDW } (\%) = \frac{DW_{60} - DW_{550}}{DW_{60}} \cdot 100 \tag{1}$$

$$CO_2\ (\%) = \frac{DW_{550} - DW_{925}}{DW_{60}} \cdot 100 \tag{2}$$

Assuming that ${CO_3}^{2-}$ was bound to calcium, it was then multiplied by 1.67 (fraction of ${CO_3}^{2-}$ in $CaCO_3$) to receive calcium carbonate equivalents. Mineral remains (= total minerals - carbonates) resulted from the subtraction of ash at 925 °C from calcium oxide, which was calculated from the CO_2 loss multiplied by 1.27 (fraction of CO_2 in CaO).

Encrustation of charophytes from hard-water lakes was referred to $CaCO_3$ content (%) of plant dry weight. For habitat-specific investigations (4.3 Age gradient and 4.4 Freshwater and brackish water habitats), encrustation was calculated to carbonate content (%) of plant DW because of other precipitated carbonates (Anadon et al. 2002; Schöler et al. 2014).

Age dependent encrustation is referred to the cell age along the plant thallus at the sampling. Therefore, plants were analysed in parts of thallus for the LOI analysis (Figure 10).

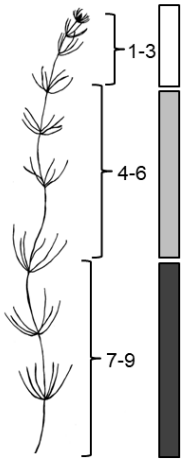

Figure 10: Encrustation of cell age along the thallus. Plants were analysed in first to third, forth to sixth, seventh to ninth whorls and internodes. Side branches were removed.

Element content

Charophyte DW was analysed by inductively coupled plasma-optic emission spectroscopy (ICP-OES) for encrustation relevant elements in plants with carboante crust and in the separate carboante crust.

Plants with carbonate crust

Ca, Fe, K, Mg, Na and P contents of plants with carbonate crust were analysed by ICP-OES. For this, a sample of 0.1 g powdered plant DW was digested with 5 ml HNO_3 (65 %) and 3 ml H_2O_2 (30 %) for 1.5 hours by microwave extraction (CEM MARS 6). The extracted samples were filled up to 25 ml with ultrapure water and were filtered (601P, Rotilabo) before measurements. Samples were analysed spectrometric for Ca (317.9 nm), Fe (238.2 nm), K (766.5 nm), Na (589.6 nm), Mg (285.2 nm) and P (214.9 nm) with an Optima 8300 spectrometer from Perkin Elmer. Element contents of Ca, Fe, K, Mg, Na, and P are presented as g kg^{-1} DW. For investigations of habitat-specificity (4.4 Freshwater and brackish water habitats) K, Na, and P contents were calculated as g kg^{-1} AFDW, because K^+ and Na^+ are main ions in the turgor regulation (Winter and Kirst 1990) and P content is mainly stored in the organic part of the plant (Kufel et al. 2013). Mg/Ca- and K/Na-ratios are calculated from individual sample weight.

Carbonate crust

Dried plant samples were weighted with analytical balance Mettler Toledo XP205 Delta Range before and after extraction in diluted 30 % HCl solution (Suprapur, Merck). The carbonate crust was dissolved for 5 hours by 50 rpm in 10 ml HCl (c = 0.0094 mol L^{-1}). The weight loss represented the carbonate crust. This method was established by Schöbe (2017) allowing for complete dissolution of the carbonate crust without damaging the plant cortex. Sampels were analysed spectrometrically for Ca (422.7 nm), Cd (228.8), Cu (327.4 nm), Fe (259.9 nm), K (766.5 nm), Mg (279.1 nm), Mn (257.6 nm), Na (589.6 nm), P (213.6 nm), Pb (220.4 nm), and Zn (213.9 nm) via ICP-OES (Optima 7300, Perkin Elmer). Elements of carbonate crust are presented as mg g^{-1}.

Photosynthesis

Photosynthesis, as a measure of the physiological state, was measured with a Pulse Amplitude Modulation (PAM) fluorometer (Diving-PAM, Walz). Plants were dark adapted for at least 15 minutes before rapid light curve measurements. Saturation flash was set every 10 seconds with a pulse length of 2.5 seconds by the PAM itself. Eight irradiation levels of 25, 97, 224, 407, 618, 864, 1266, and 1769 µmol m^{-2} s^{-1} were applied. Quantum yield, the state of excitation energy in photosystem II, was calculated by the PAM software PamWin.

Relative electron transport rates (ETR) of photosystem II were calculated using the model of Eilers and Peeters (1988). ETR_{max} was expressed with the parameters a, b, and c to adjust the production curve (Equations 3 and 4). The parameters depend on s = tan(ϕ) of initial slope and I_m = optimal intensity.

$$\mathrm{ETR_{max}}\ (\mu\mathrm{mol\ electrons\ m^{-2}\ s^{-1}}) = \frac{1}{b + 2\sqrt{a \cdot c}} \tag{3}$$

$$\mathrm{a} = \frac{1}{\mathrm{sI_m}^2}; \qquad \mathrm{b} = \frac{1}{\mathrm{ETR_{max}}} - \frac{2}{\mathrm{sI_m}^2}; \qquad \mathrm{c} = \frac{1}{\mathrm{s}} \tag{4}$$

Pigment content

Photosynthetic parameters were based on chlorophyll. Therefore, pigment content was extracted with 3 ml of N, N-dimethylformamide and samples were incubated overnight at 4 °C. Extinction (E) at 470, 647, 664, and 750 nm was measured using a Specord M42 spectrophotometer (Carl Zeiss Jena, Germany) and pigment contents of chlorophyll (chl) *a*, *b*, and carotenoid (car) were calculated (Equations 5, 6, and 7) after Porra et al. (1989) and Küster et al. (2004).

$$\text{chl } a\ (\mu\mathrm{g\ mL^{-1}}) = (12 \cdot (\mathrm{E_{664}} - \mathrm{E_{750}})) - (3.11 \cdot (\mathrm{E_{647}} - \mathrm{E_{750}})) \tag{5}$$

$$\text{chl } b\ (\mu\mathrm{g\ mL^{-1}}) = (20.78 \cdot (\mathrm{E_{647}} - \mathrm{E_{750}})) - (4.88 \cdot (\mathrm{E_{664}} - \mathrm{E_{750}})) \tag{6}$$

$$\text{carotenoid}\ (\mu\mathrm{g\ mL^{-1}}) = \frac{(1000 \cdot \mathrm{E_{470}}) - (3.27 \cdot \text{chl } a) - (104 \cdot \text{chl } b)}{229} \tag{7}$$

Cultivation

For the heavy metal exposure experiment, charophytes were cultivated to allow consistent conditions for measurements. Water tank was adapted by means of a thermostat (OR 3500) to a temperature of 15 °C. Light was supplied by fluorescent lamps (n = 5, Phillips TLD 36W/950) on a 12 h/12 h light-dark cycle. Photon flux density was measured with a light meter (LI-250, LI-COR) and set to 50-80 µmol photons m^{-2} s^{-1}. Water and sediment samples were taken in the Schlüsselstollen, in the river Saale above and below the Schlenze discharges into the Saale, and in the Altarm of the river Saale. Further samples, including plant material, were collected in two habitats Asche (H1) and Lützlower See (H2). *C. tomentosa* was found in both habitats whereas *C. subspinosa* occurred only in Lützlower See. Charophytes were planted in beakers (0.5 L) with 70 g sediment and filled up to 0.4 L with water. Experiment was conducted for ten days according to Table 2.

Table 2: Experimental approaches for incubation with sediment and water from Asche, Lützlower See, $Saale_{above}$ and $Saale_{below}$ the Schlenze estuary, Altarm, and Schlüsselstollen. Altarm:Schlüsselstollen water was mixed in a volume ratio of 3:1. Plants were incubated in different sediment/water combinations. For abbreviation of approaches compare table.

		sediment					
		Asche	Lützlower See	$Saale_{above}$	$Saale_{below}$	Altarm	Schlüsselstollen
water	Asche	H1H1					
	Lützlower See		H2H2				
	$Saale_{above}$			SaSa			
	$Saale_{below}$				SbSb		
	Altarm					AA	
	Altarm/Schlüssel-stollen (3:1)	H1AS	H2AS				
	Schlüsselstollen	H1S	H2S				SS

Absorption of heavy metals from water was measured with ICP-OES (Optima 7300, Perkin Elmer) as mentioned above. Adsorption was calculated as the concentration difference before and after incubation of plants for ten days (4.5 Heavy metal exposure).

3.2.2 Water samples

Water chemistry of investigated sampling sites was analysed. Conductivity (cond) and pH of the surface water were measured directly at the sites with a HACH HQ40d (Hach-Lange, Germany). Water samples were collected in 50 ml tubes for the analysis of Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{-}, $SO_4{}^{2-}$, and total inorganic carbon at the Helmholtz Centre for Environmental Research (UFZ) – Central Laboratory for Water Analytics and Chemometrics. For quantitative analysis of main cation and anion concentrations, a Dionex ICS 3000 ion chromatograph with suppressor technique for both ion types was used. TIC was determined with a Dimatoc 2000. According to the lower level of quantification and calibrated range, some samples with relatively low conductivity were measured undiluted. The samples with higher salinity were diluted up to 1:100 with high purity water before the measurements. All measurements were conducted in accordance to EN ISO 14911:1999 and EN ISO 10304:2009-1. For total phosphorus (TP), water samples were collected in polypropylene tubes cleaned with 10 % hydrochloric acid and ultrapure water. TP concentration was determined in duplicates after alkaline persulphate oxidation as described by Koroleff (1983) in the laboratory.

3.3 Statistical analyses

Statistical analyses were conducted with R (R Core Team 2017). Data were analysed for normality and homogeneity by means of Shapiro-Wilk and Levene test (Fox and Weisberg 2011). In case of normal and homogeneous distribution, data were analysed with an analysis of variance (ANOVA) and Tukey Honestly Significant Difference (HSD) post-hoc test. Non-normally distributed data were analysed with the Kruskal-Wallis test and Fisher Least Significant Difference (LSD) post-hoc test. The p-values were adjusted by Bonferroni correction to identify significant differences (de Mendiburu 2016). The relationship of two variables was tested by means of Pearson (parametric) or Spearman (non-parametric) correlations test (Oksanen et al. 2017). Multivariate analyses were performed with principal component analysis (PCA), based on standardised plant data, and non-metric multidimensional scaling (NMDS) (Oksanen et al. 2017). Therefore, plant data were transformed (Bray-Curtis) and plotted in an NMDS. Standardised water chemistry data were fitted to ordination. Significant water chemistry parameters were identified with a permutation test (number of permutations = 999). Significance levels were set to a p-value < 0.05.

4 Results

4.1 Seasonality

Encrustation and element composition of charophyte species were studies in two hard-water lakes (Krüselinsee and Lützlower See) for a period of one year.

Water chemistry

Water chemistry was significantly different in Krüselinsee and Lützlower See, with higher ion concentrations of Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{+}, $SO_4{}^{2-}$, and TIC exhibited in Lützlower See (Figure 11 and Table 3). The greatest ion differences were of Cl^{-} and $SO_4{}^{2-}$ concentrations. Ca^{2+} concentration was 2.6 fold higher in Lützlower See than in Krüselinsee. In both lakes, the lowest Ca^{2+} and TIC concentrations were measured in summer months. No significant difference in pH was found for both study sites.

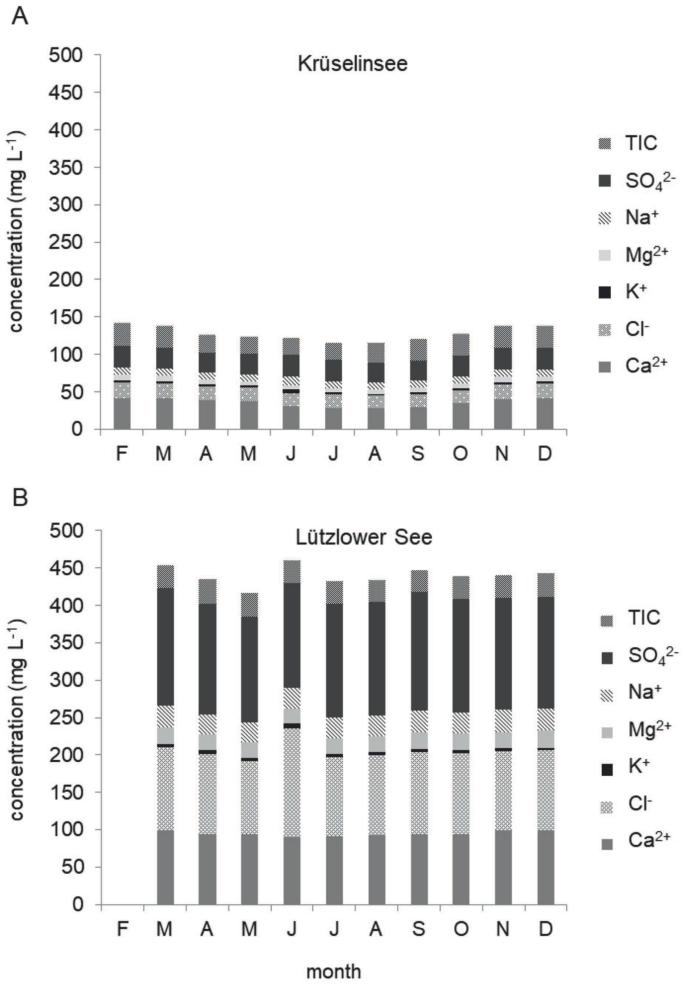

Figure 11: Monthly concentration of Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{+}, $SO_4{}^{2-}$, and TIC (mg L^{-1}) in Krüselinsee (A) and Lützlower See (B). Months are abbreviated as first letter.

Table 3: Comparison of water chemistry data of Krüselinsee and Lützlower See (mean ± SD), n = number of samplings. Listed are Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{+}, $SO_4{}^{2-}$, and TIC concentrations (mg L^{-1}), pH, and conductivity (mS cm^{-1}). Data were taken monthly from April 2016 – March 2017. Different letters indicate significant differences (Fisher LSD post-hoc test, $p < 0.05$) between Krüselinsee and Lützlower See for the given parameter.

	Krüselinsee	Lützlower See
n	11	10
Ca^{2+}	36.9 ± 5.5 a	95.0 ± 3.6 b
Cl^{-}	18.6 ± 1.3 a	111 ± 13.0 b
K^{+}	2.7 ± 0.6 a	4.2 ± 0.9 b
Mg^{2+}	6.6 ± 0.4 a	21.7 ± 0.7 b
Na^{+}	9.6 ± 0.7 a	28.1 ± 1.1 b
$SO_4{}^{2-}$	27.9 ± 1.0 a	150 ± 5.8 b
TIC	26.6 ± 3.2 a	30.8 ± 1.3 b
pH	8.3 ± 0.5 a	8.4 ± 0.4 a
cond	0.32 ± 0.05 a	0.82 ± 0.04 b

Seasonal pattern of encrustation

The encrustation, proportion of $CaCO_3$ of plant dry weight, is shown for investigated charophytes from Krüselinsee (Figure 12) and Lützlower See (Figure 13). Encrustation increased during spring, reached maxima over the summer and declined in autumn and winter. This pattern was observed for *C. contraria*, *C. subspinosa* and *Nitellopsis obtusa* in Krüselinsee and *C. globularis* in Lützlower See. Precipitated $CaCO_3$ of *C. tomentosa* from Lützlower See increased from April to September. A decrease in encrustation of *C. tomentosa* was not observed until October (Figure 13C). No seasonal pattern of encrustation was found for *C. tomentosa* in Krüselinsee (Figure 12C) and for *C. subspionsa* in Lützlower See (Figure 13B). The highest encrustation was detected for *C. contraria* (in June: 74.8 % ± 0.8 %) and for *N. obtusa* (in August: 74.9 % ± 1.9 %) in Krüselinsee (Figures 12A and D). The lowest precipitated $CaCO_3$ was found in Krüselinsee for *C. subspinosa* (in March: 56.1 % ± 1.2 %), which germinated in spring 2017 (Figure 12B, hashtag) and for *N. flexilis*/*opaca* (in April: 51.3 % ± 1.4 %) in Lützlower See. *Nitella flexilis*/*opaca* was only found for three months (Figure 13D).

Species-specificity was analysed for *C. contraria*, *C. subspinosa*, *C. tomentosa*, and *Nitellopsis obtusa* from Krüselinsee (Table 4) and for *C. globularis*, *C. subspinosa*, *C. tomentosa*, and *N. flexilis*/*opaca* from Lützlower See (Table 5). In both lakes, all species precipitated $CaCO_3$ in a species-specific pattern (Fisher LSD post-hoc test, $p < 0.05$).

Habitat-specificity was analysed for *C. subspinosa* and *C. tomentosa* growing in Krüselinsee and Lützlower See (Table 6). Both species exhibited significantly higher encrustation in Lützlower See than in Krüselinsee (Tukey HSD post-hoc test, $p < 0.05$). In July and August, no significant difference was found for the encrustation of both species in Krüselinsee and Lützlower See (Tukey HSD post-hoc test, $p \geq 0.05$).

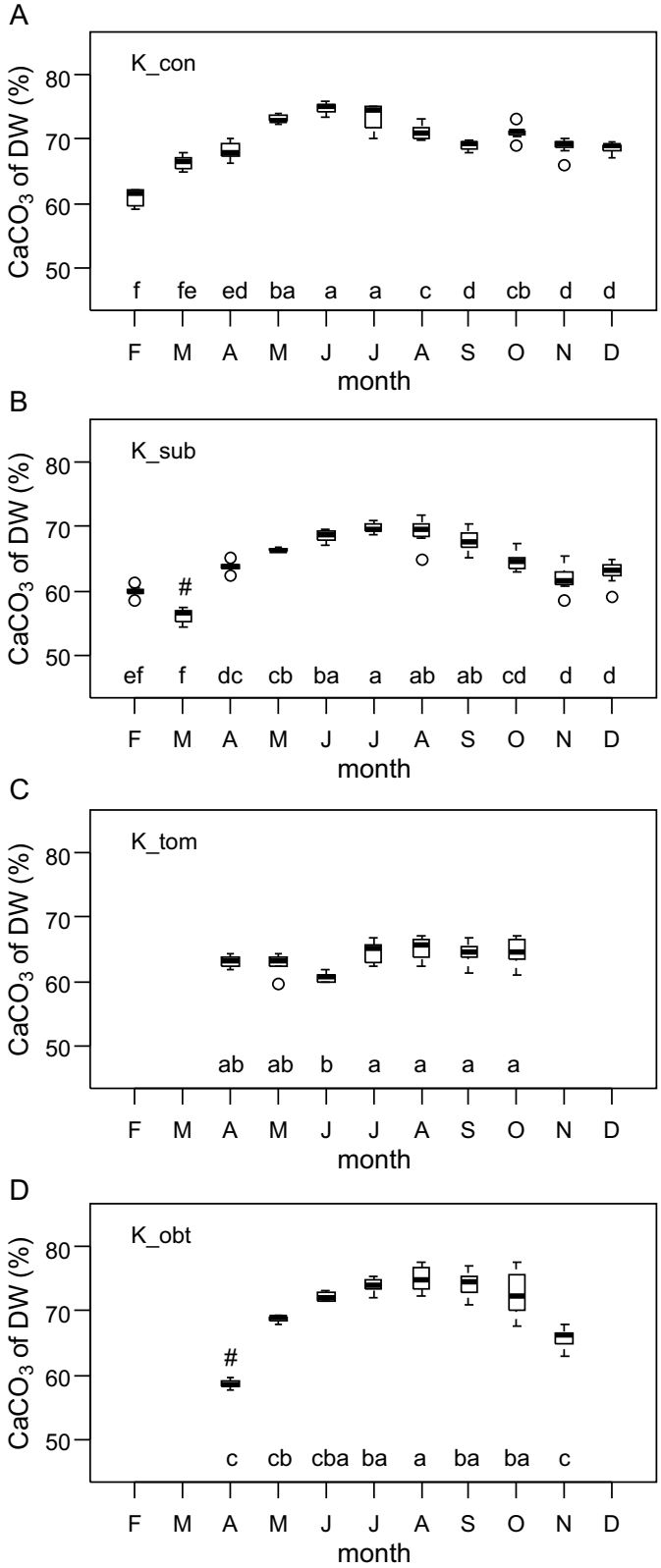

Figure 12: The month-to-month variation of $CaCO_3$ content based on DW (%) of *C. contraria* (con) (A), *C. subspinosa* (sub) (B), *C. tomentosa* (tom) (C), and *Nitellopsis obtusa* (obt) (D) from Krüselinsee. Sample size was n ≥ 6, except for *N. obtusa* in April and May, where only three samples were analysed. Box plots include whiskers (5-95 % of variability) and outliers (points). Hashtags show the new generation of plants, which germinated recently before sampling. Different letters indicate significant difference (Fisher LSD post-hoc test, $p < 0.05$) in encrustation. Months are abbreviated as first letter.

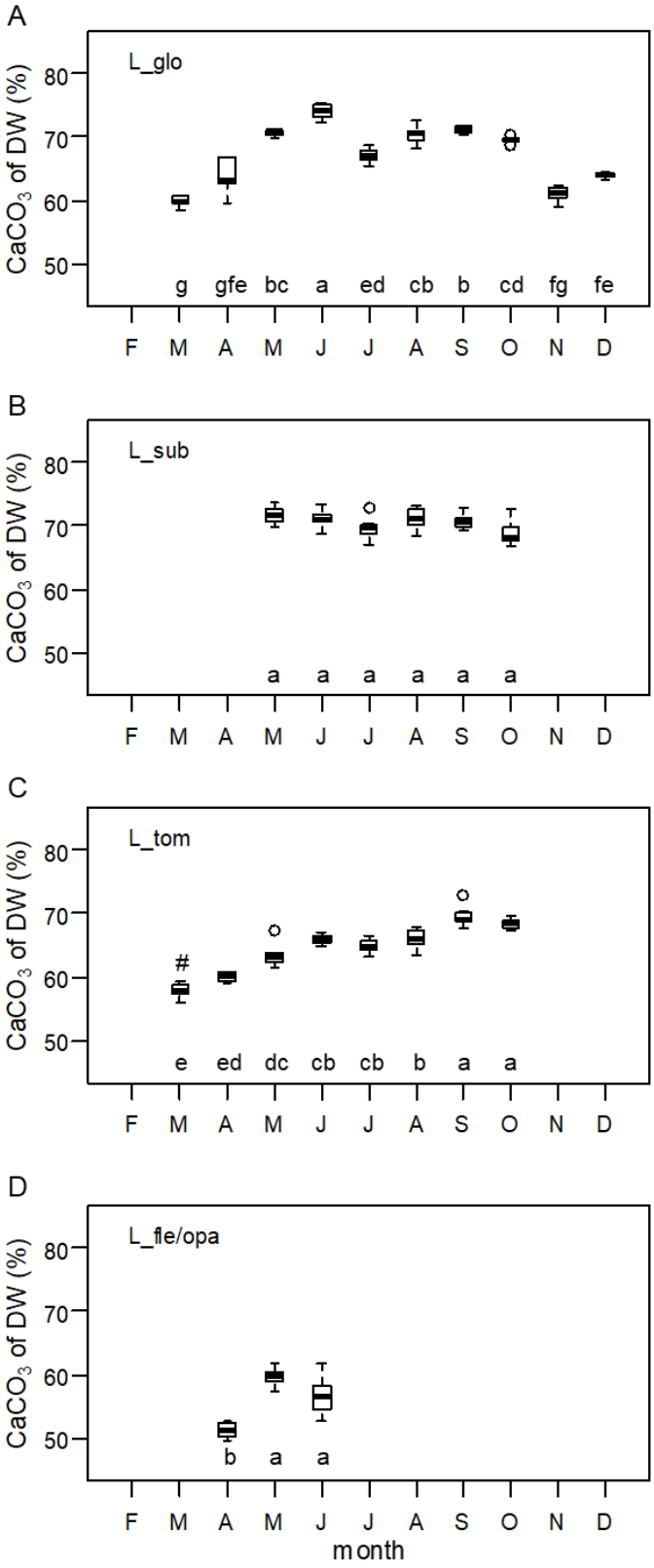

Figure 13: The month-to-month variation of $CaCO_3$ content based on DW (%) of *C. globularis* (glo) (A), *C. subspinosa* (sub) (B), *C. tomentosa* (tom) (C), and *N. flexilis*/*opaca* (fle/opa) (D) from Lützlower See. Sample size was $n \geq 6$. Box plots include whiskers (5-95 % of variability) and outliers (points). Hashtag shows the new generation of plants, which germinated recently before sampling. Different letters indicate significant difference (Fisher LSD post-hoc test, $p < 0.05$) in encrustation. Months are abbreviated as first letter.

Table 4: Comparison of $CaCO_3$ content (mean ± SD) based on DW (%) of *C. contraria*, *C. subspinosa*, *C. tomentosa*, and *Nitellopsis obtusa* from Krüselinsee (K). Months are abbreviated as first letters. Different letters indicate species-specific significant difference (Fisher LSD post-hoc test, $p < 0.05$).

month	K_con	K_sub	K_tom	K_obt
F	61.0 ± 1.3 a	59.9 ± 0.9 a		
M	66.4 ± 1.1 a	56.1 ± 1.2 b		
A	68.1 ± 1.4 a	63.8 ± 1.0 b	63.1 ± 1.0 bc	58.7 ± 0.8 c
M	73.2 ± 0.6 a	66.4 ± 0.3 c	62.8 ± 1.7 d	68.8 ± 0.7 b
J	74.8 ± 0.8 a	70.0 ± 2.0 c	62.7 ± 3.1 d	72.2 ± 0.7 b
J	73.3 ± 2.1 a	71.1 ± 3.3 b	62.1 ± 3.3 c	74.0 ± 1.2 a
A	71.0 ± 1.0 b	69.1 ± 2.2 b	65.2 ± 1.8 c	74.9 ± 1.9 a
S	69.0 ± 0.7 b	67.8 ± 1.8 b	64.9 ± 1.8 c	74.1 ± 2.1 a
O	71.0 ± 1.1 a	64.6 ± 1.3 b	64.6 ± 2.1 b	72.7 ± 3.6 a
N	68.9 ± 1.4 a	61.9 ± 2.1 b		65.7 ± 1.7 c
D	68.6 ± 0.9 a	62.9 ± 2.0 b		

Table 5: Comparison of $CaCO_3$ content (mean ± SD) based on DW (%) of *C. subspinosa*, *C. tomentosa*, and *N. flexilis/opaca* from Lützlower See (L). Months are abbreviated as first letters. Different letters indicate species-specific significant difference (Fisher LSD post-hoc test, $p < 0.05$).

month	L_glo	L_sub	L_tom	L_fle/opa
M	59.9 ± 0.8 a		57.9 ± 1.2 b	
A	63.8 ± 3.0 a		60.0 ± 0.8 a	51.3 ± 1.4 b
M	70.6 ± 0.6 a	71.7 ± 1.4 a	63.5 ± 2.0 b	59.7 ± 1.4 c
J	74.1 ± 1.2 a	70.5 ± 1.4 b	64.1 ± 2.4 c	56.8 ± 3.1 d
J	67.2 ± 1.2 b	70.5 ± 1.9 a	63.5 ± 2.8 c	
A	70.3 ± 1.5 a	71.2 ± 1.6 a	66.0 ± 1.5 b	
S	71.1 ± 0.6 a	70.7 ± 1.2 ab	69.5 ± 1.6 b	
O	69.6 ± 0.5 a	68.8 ± 2.1 a	68.4 ± 0.9 a	
N	61.1 ± 1.2			
D	64.1 ± 0.5			

Table 6: Comparison of $CaCO_3$ content (mean ± SD) based on DW (%) of *C. subspinosa* (sub) and *C. tomentosa* (tom) from Krüselinsee (K) and Lützlower See (L). Months are abbreviated as first letters. Different letters indicate habitat-specific significant difference (Tukey HSD post-hoc test, $p < 0.05$).

	sub		tom	
	K	L	K	L
A			63.1 ± 1.0 A	60.0 ± 0.8 B
M	66.4 ± 0.3 A	71.7 ± 1.4 B	62.8 ± 1.7 A	63.5 ± 2.0 A
J	70.0 ± 2.0 A	70.5 ± 1.4 A	62.7 ± 3.1 A	64.1 ± 2.4 B
J	71.1 ± 3.3 A	70.5 ± 1.9 A	62.1 ± 3.3 A	63.5 ± 2.8 A
A	69.1 ± 2.2 A	71.2 ± 1.6 A	65.2 ± 1.8 A	66.0 ± 1.5 A
S	67.8 ± 1.8 A	70.7 ± 1.2 B	64.9 ± 1.8 A	69.5 ± 1.6 B
O	64.6 ± 1.3 A	68.8 ± 2.1 B	64.6 ± 2.1 A	68.4 ± 0.9 B
∑	67.6 ± 2.3 A	70.5 ± 1.8 B	63.7 ± 2.1 A	65.7 ± 3.2 B

Correlation of precipitated $CaCO_3$

Correlations of encrustation with water chemistry and element contents of plant DW are shown in Table 7. In Krüselinsee, significant correlations between precipitated $CaCO_3$ and the water chemistry parameters were found for all parameters except Na^+ concentration. Ca^{2+} and TIC concentrations correlated significantly with the encrustation of plants from Lützlower See. For both lakes, a similar positive correlation was found between Ca content of plants DW and precipitated $CaCO_3$. Fe and P contents correlated negatively with the encrustation in Krüselinsee and Lützlower See, whereas the K content only correlated significantly in Lützlower See.

Table 7: Significant correlations of precipitated $CaCO_3$ of plant DW (%) with water chemistry and biomass element content within lakes and species from Krüselinsee (K) and Lützlower See (L). Species are abbreviated as first three letters. Differences were tested with Spearman correlation test, p-values with: * < 0.05, ** < 0.01, *** < 0.001.

		habitat-specific		species-specific						
		K	L	K_con	K_sub	K_tom	K_obt	L_glo	L_sub	L_tom
water chemistry	pH	0.40***		0.60***	0.72***			0.35**		
	cond	-0.33***			-0.41***	-0.56***	-0.54***	-0.72***		
	Ca^{2+}	-0.53***	-0.35***	-0.69***	-0.90***		-0.57***			-0.28*
	Cl^-	-0.40***		-0.53***	-0.70***					
	K^+	-0.27***			-0.47***	-0.57***	-0.48**			
	Mg^{2+}	-0.40***		-0.53***	-0.76***			-0.37**		0.46**
	Na^+							-0.49***		
	$SO_4{}^{2-}$	-0.16*			-0.25*					0.27*
	TIC	-0.51***	-0.48***	-0.58***	-0.80***		-0.57***	-0.50***		-0.60***
biomass	Ca	0.85***	0.83***	0.93***	0.67***		0.48***	0.83***		
	Fe	-0.29***	-0.45***	-0.66***	-0.80***	-0.41**	-0.60***	-0.78***	-0.34*	
	K		-0.20**		0.37**			0.32*	0.50***	
	Mg				0.80***		0.42**		0.37*	-0.47***
	P	-0.52***	-0.42***	-0.76***				-0.53***		-0.38***

Within Krüselinsee, species-specific correlations were found. *C. contraria* and *C. subspinosa* exhibited a pattern of correlations and significances almost similar to the habitat-specific analysis presented before. However, encrustation of both species correlated stronger with the pH than all four species on average in Krüselinsee. The precipitated $CaCO_3$ content of *C. contraria* was insignificantly dependent on conductivity, K^+, Na^+, and $SO_4{}^{2-}$ concentrations of the water. The encrustation of *C. tomentosa* correlated only with the conductivity and K^+ concentration significantly. The same was true for *Nitellopsis obtusa*, which also exhibited a significant correlation with the Ca^{2+} and TIC concentrations. Comparing the significant values between the species, the Ca^{2+} and TIC concentrations dependency of the encrustation was stronger for *C. subspinosa* compared to *C. contraria* and *Nitellopsis obtusa*. The significant correlations of the precipitated $CaCO_3$ and the K^+ concentration were similar between *C. subspinosa*, *C. tomentosa* and *N. obtusa*.

In Lützlower See, precipitated $CaCO_3$ of *C. globularis* correlated significantly with the water chemistry parameters including Na^+ concentration. The encrustation of *C. subspinosa* showed no correlation to the water chemistry, whereas the precipitated $CaCO_3$ of *C. tomentosa* correlated significantly to Ca^{2+}, Mg^{2+}, $SO_4{}^{2-}$ and TIC concentrations. However, the correlation with Mg^{2+} and $SO_4{}^{2-}$ concentrations was positive for *C. tomentosa*, which was in contrast to the other species. No test was performed for *N. flexilis/opaca* for three sampling months.

A number of significant correlations between encrustation and element contents of plants from Krüselinsee and Lützlower See were found. Encrustation of *C. contraria* and *C. globularis* correlated similarly to habitat-specific analyses. Precipitated $CaCO_3$ of *C. subspinosa* correlated with Fe, K, and Mg contents in both lakes and also with Ca content in Krüselinsee. *C. tomentosa* exhibited habitat-specific correlation; in Krüselinsee with Fe and in Lützlower See with Mg and TP contents. *N. obtusa* exhibited significant correlations with Ca, Fe, and Mg contents in Krüselinsee.

Plant element content

Plant element content of Ca, Fe, K, Mg, and P is shown in Table 8. Species-specific element content was tested within both Krüselinsee and Lützlower See. Habitat-specific element content was analysed for species (*C. subspinosa* and *C. tomentosa*) occurring in both lakes. *C. contraria* differed from *C. tomentosa* with the highest Ca content based on plant

Table 8: Comparison of plant element composition (mean ± SD) of charophytes from Krüselinsee (K) and Lützlower See (L). Element contents of Ca, Fe, K, Mg, and TP are presented as g kg^{-1} DW. Species are abbreviated as first three letters. Different small/capital letters indicate species/habitat-specific significant difference (Fisher LSD post-hoc test, $p < 0.05$).

species	n	Ca	Fe	K	Mg	P
K_con	11	296 ± 20 a	0.07 ± 0.05 a	3.5 ± 0.6 c	3.4 ± 0.3 b	0.7 ± 0.2 a
K_sub	11	270 ± 16 ab, A	0.06 ± 0.06 a, A	7.6 ± 1.9 b, A	4.2 ± 0.4 a, A	1.0 ± 0.3 a, A
K_tom	7	257 ± 21 b, C	0.03 ± 0.01 a, B	10.7 ± 4.2 ab, B	3.6 ± 0.4 ab, C	0.9 ± 0.3 a, C
K_obt	7	283 ± 22 ab	0.08 ± 0.08 a	19.4 ± 4.2 a	3.6 ± 0.3 ab	0.8 ± 0.4 a
L_glo	10	285 ± 22 ab	0.21 ± 0.09 a	7.0 ± 1.6 b	4.7 ± 0.7 a	1.2 ± 0.2 a
L_sub	6	294 ± 5 a, B	0.07 ± 0.02 a, A	6.8 ± 0.5 b, A	4.7 ± 0.3 a, B	0.6 ± 0.1 b, B
L_tom	8	265 ± 19 bc, C	0.08 ± 0.04 a, C	10.8 ± 2.9 a, B	4.8 ± 0.6 a, D	1.0 ± 0.3 ab, C
L_fle	3	225 ± 18 c	0.41 ± 0.16 a	8.5 ± 5.8 ab	4.3 ± 0.6 a	1.2 ± 0.6 ab

DW in Krüselinsee. In Lützlower See *C. subspinosa* had the highest and *N. flexilis/opaca* had the lowest Ca content in plant DW. *C. subspinosa* differed in Ca contents in Krüselinsee and Lützlower See, whereas *C. tomentosa* exhibited no habitat-specific difference. Fe contents were similar for species but *C. globularis* and *N. flexilis/opaca* exhibited higher contents. Habitat-specific Fe contents were found for *C. tomentosa*. K content was lowest for *C. contraria* and highest for *Nitellopsis obtusa* in Krüselinsee. In Lützlower See, K content of *C. tomentosa* differed significantly from *C. globularis* and *C. subspinosa*. Mg

content of *C. subspinosa* was significantly higher compared to *C. contraria* in Krüselinsee. No difference in Mg content was found for species in Lützlower See. Habitat-specific Mg contents were found for *C. subspinosa* and *C. tomentosa*. For the P content, *C. subspinosa* in Lützlower See differed significantly from *C. globularis* and was also habitat-specific for *C. subspinosa*.

Interaction of investigated parameters

The NMDS plot (dimensions = 2, stress = 0.04) shows site-, species-, and season-specific element contents based on plant dry weight (Figure 14). For this analysis, the sampling dates were grouped along two dimensional lines: spring–autumn (SA) and summer–winter (SW). Water chemistry parameters and the sampling dates were fitted to the plot. Concentration of TIC and seasonality parameters, both dimensions tested, showed a significant impact on the element content distribution of investigated species ($p < 0.05$).

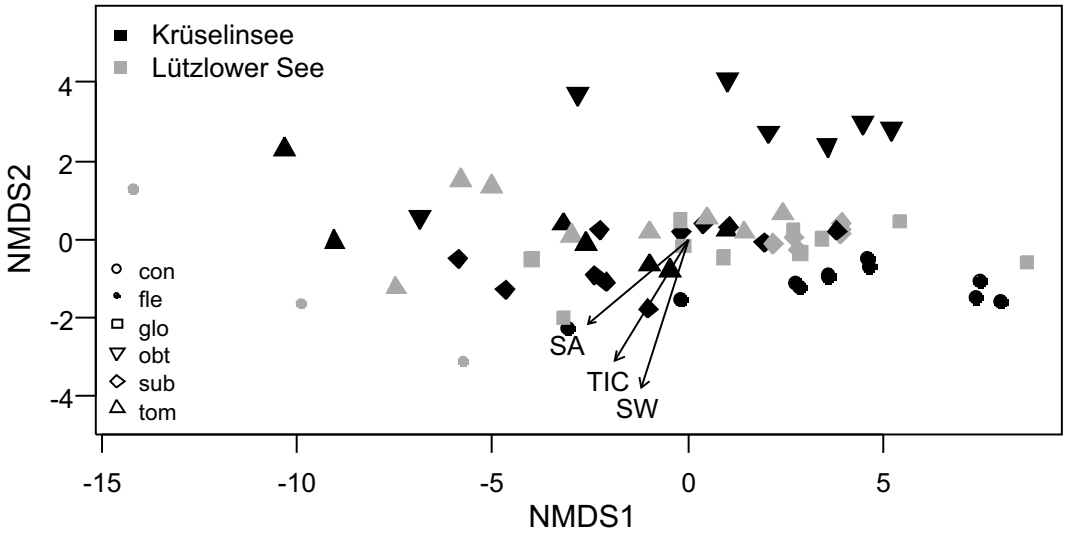

Figure 14: NMDS plot of the element composition of Ca, Fe, K, Mg, and P (mean of months) of charophytes from Krüselinsee (black) and Lützlower See (grey) (dimensions = 2, stress = 0.04). Species are represented by different symbols, see legend. The concentration of total inorganic carbon (TIC) and seasonal dimensions (SA, spring – autumn; SW, summer – winter) were fitted as vector to plot ($p < 0.05$).

4.2 Fertility

The influence of fertility on encrustation was investigated for *C. subspinosa* and *C. tomentosa* from Krüselinsee and Lützlower See in June and July 2016. Habitat-specific encrustation was tested for both species. *C. subspinosa* and *C. tomentosa* exhibited no difference in encrustation between the lakes (ANOVA, $p \geq 0.05$). Therefore habitat-specific influence was negligibly for the analysis of encrustation.

In June, $CaCO_3$ content of fertile and non-fertile plants is shown in Figure 15A and Table 9. Encrustation was higher for fertile and non-fertile plants of *C. subspinosa* compared to *C. tomentosa*. Differences in precipitated $CaCO_3$ of fertile and non-fertile plants were found for *C. tomentosa* exhibiting higher encrustation in non-fertile plants. For *C. subspinosa*, encrustation was not influenced significantly of the state of maturity.

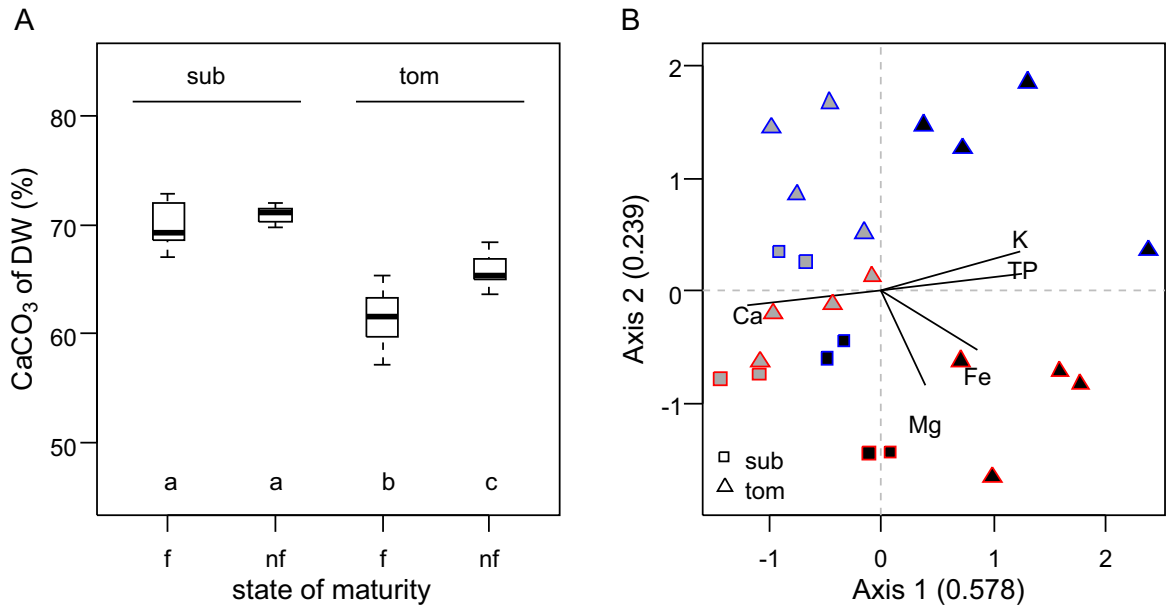

Figure 15: A. Comparison of $CaCO_3$ of DW (%) of fertile (f) and non-fertile (nf) *C. subspinosa* (sub) and *C. tomentosa* (tom) from Krüselin See and Lützlower See combined togehter. Box plots include whiskers (5-95 % of variability). Different letters indicate significant difference among groups (Tukey HSD post-hoc test, $p < 0.05$). B. PCA plot of element contents (Ca, Fe, K, Mg, and P) of charophytes from Krüselinsee (blue) and Lützlower See (red). *C. subspinosa* and *C. tomentosa* were plotted with different symbols, see legend. Background of symbols indicate fertile (black) and non-fertile (grey) state of maturity.

Element contents (Ca, Fe, K, Mg, and P) of fertile and non-fertile plants of *C. subspinosa* and *C. tomentosa* were plotted (Figure 15B). Habitat-specific element content was found (Appendix Table A1); plants from Lützlower See had higher contents of Mg and Fe in plant DW (Tukey HSD post-hoc test, $p < 0.05$). Fertile and non-fertile plants were separated by the axis 1 which was determined by the contents of P, K, and Ca. These element contents differed in *C. tomentosa*; fertile plants had lower Ca and higher K and P contents than non-fertile plants (Table 9). In *C. subspinosa* no significant difference in element content

was observed. Part of thalli, divided into whorls and internodes, of fertile *C. subspinosa* and *C. tomentosa* were analysed in July (Figure 16 and Table 10). $CaCO_3$ content was higher in whorls than in internodes of *C. subspinosa* and female *C. tomentosa*. Male *C. tomentosa* encrusted independently of part of thalli (Figure 16A).

Element content was tested for habitat-specificity; exhibiting higher Fe and Mg contents in species from Lützlower See (Appendix Table A2). Ca, Fe, K, Mg, and P contents of whorls and internodes of plant thalli are shown in Figure 16B and Table 16. The PCA plot showed a distribution along axis 1 for *C. subspinosa*, which was described by the contents of Ca, Mg, and K. Samples of *C. tomentosa* were distributed along both axes and part of thalli were mainly separated by axis 2 (Fe, Mg, and P contents). In detail, element contents are listed in Table 10. Ca contents were higher in male *C. tomentosa* internodes than whorls, opposite trend was observed in female *C. tomentosa* and *C. subspinosa*. K contents of *C. tomentosa* internodes were increased in comparison to whorls, whereas whorls had higher P contents. Element contents of *C. subspinosa* showed no significant difference in part of thalli.

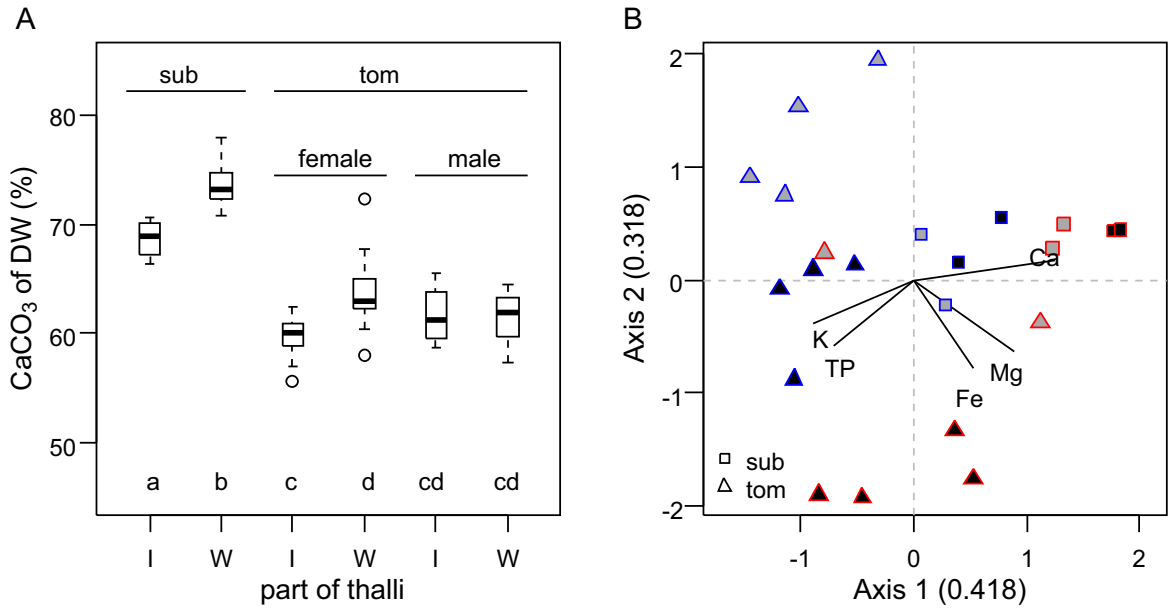

Figure 16: A. Comparison of $CaCO_3$ based on DW (%) of internode (I) and whorl (W) of *C. subspinosa* (sub) and *C. tomentosa* (tom, female and male plants) from Krüselin See and Lützlower See combined togehter. Different letters indicate significant difference among groups (Tukey HSD post-hoc test, $p < 0.05$). Box plots include whiskers (5-95 % of variability) and outliers (points). B. PCA plot of element contents (Ca, Fe, K, Mg, and P) of charophytes from Krüselinsee (blue) and Lützlower See (red). *C. subspinosa* and *C. tomentosa* were plotted with different symbols, see legend. Background of symbols indicate part of thallus (internode = grey, whorl = black).

Table 9: Comparison of $CaCO_3$ based on DW (%) and element contents (mean ± SD) of Ca, Fe, K, Mg, and P (g kg^{-1}) of fertile (f) and non-fertile (nf) of *C. subspinosa* (sub) and *C. tomentosa* (tom). Different letters indicate significant difference (Tukey HSD post-hoc test, $p < 0.05$) for the given parameters, n = number of replicates for LOI analysis.

sample	n	$CaCO_3$	Ca	Fe	K	Mg	P	$CaCO_3$/P
sub_f	7	69.9 ± 1.6 a	270.1 ± 9.6 a	0.07 ± 0.05 a	8.5 ± 0.3 a	4.9 ± 0.1 a	0.68 ± 0.07 a	103.2 ± 7.6 ab
sub_nf	8	71.0 ± 0.2 a	294.1 ± 15.2 a	0.03 ± 0.02 a	7.6 ± 0.9 a	4.4 ± 0.2 ab	0.50 ±0.22 a	159.0 ± 70.6 b
tom_f	17	61.7 ± 1.4 b	227.4 ± 11.5 b	0.10 ± 0.07 a	12.6 ± 1.5 b	4.5 ± 0.7 a	1.16 ± 0.36 b	57.5 ± 18.7 a
tom_nf	10	65.7 ± 1.1 c	266.5 ±25.5 a	0.05 ± 0.03 a	8.5 ± 1.5 a	3.8 ± 0.4 b	0.48 ± 0.08 a	139.3 ± 23.1 b

Table 10: Comparison of $CaCO_3$ based on DW (%) and element contents (mean ± SD) of Ca, Fe, K, Mg, and P (g kg^{-1}) of internode (I) and whorl (W) of *C. subspinosa* (sub) and *C. tomentosa* (tom) of female and male plants. Different letters indicate significant difference (Tukey HSD post-hoc test, $p < 0.05$) for the given parameters, n = number of replicates for LOI analysis.

sample	n	$CaCO_3$	Ca	Fe	K	Mg	P	$CaCO_3$/P
sub_I	14	68.6 ± 1.5 a	279.4 ± 10 a	0.04 ± 0.01 a	7.7 ± 0.7 ab	4.3 ± 0.3 a	0.60 ± 0.26 ab	134.6 ± 74.0 a
sub_W	16	73.6 ± 1.8 b	289.0 ± 6.5 a	0.04 ± 0.03 a	6.4 ± 2.3 a	4.3 ± 0.1 a	0.54 ± 0.20 a	151.1 ± 63.7 a
tom_I_{female}	13	59.7 ± 1.9 c	235.3 ± 4.6 bc	0.01 ± 0.02 a	11.2 ± 0.1 b	3.6 ± 0.2 a	0.46 ± 0.02 a	99.8 ± 40.8 a
tom_W_{female}	14	63.8 ± 3.5 d	253.0 ± 5.6 bd	0.07 ± 0.04 a	8.4 ± 1.1 b	4.2 ± 0.6 a	0.97 ± 0.20 bc	75.2 ± 16.6 a
tom_I_{male}	8	61.7 ± 2.5 cd	259.5 ± 16.3 d	0.02 ± 0.02 a	10.2 ± 1.8 ab	3.6 ± 1.3 a	0.38 ± 0.06 a	132.5 ±16.2 a
tom_W_{male}	13	61.4 ± 2.4 cd	229.7 ± 4.4 c	0.05 ± 0.02 a	7.4 ± 0.5 ab	3.5 ± 0.3 a	1.33 ± 0.00 c	49.0 ± 5.9 a

4.3 Age gradient

Encrustation of cell age was analysed for five species from Angersdorfer Teiche, Asche, Bruchwiesen, Krüselinsee, and Lützlower See.

Water chemistry

Water chemistry data of investigated sites are listed in Table 11. The sampling sites were distinct with respect to water chemistry exhibiting enhanced ion concentrations in Angersdorfer Teiche, Asche, and Bruchwiesen. Highest ion concentrations of Ca^{2+}, Cl^{-}, K^{+}, Na^{+}, and $SO_4{}^{2-}$ were found in the gips pit Angersdorfer Teiche with exception of Mg^{2+} concentration obtaining highest concentration in Asche. Asche is located next to a potash mining heap in Teutschenthal. Bruchwiesen, a karstic spring in Bad Tennstedt, had high Ca^{2+} and $SO_4{}^{2-}$ concentrations compared to Krüselinsee and Lützlower See. Both hard-water lakes had the lowest conductivity of investigated waters. pH was around 9 in Angersdorfer Teiche, Krüselinsee and Lützlower See, 7.9 in Asche, and lowest in Bruchwiesen with 7.4.

Table 11: Water chemistry data of investigated sampling sites. Listed are Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{+}, $SO_4{}^{2-}$, TIC concentrations (mg L^{-1}), pH, conductivity (mS cm^{-1}), and salinity.

site	Ca^{2+}	Cl^{-}	K^{+}	Mg^{2+}	Na^{+}	$SO_4{}^{2-}$	TIC	pH	cond	salinity
Angersdorfer Teiche	660	2390	82.5	455	1560	2550	15.7	9.1	10.2	6.3
Asche	431	472	33.7	462	626	2380	38.1	7.9	5.0	3.0
Bruchwiesen	276	40.7	3.0	51.2	22.8	577	64.1	7.4	1.4	0.6
Krüselinsee	29.4	18.2	2.4	6.2	9.3	28.5	22.2	9.3	0.3	0.0
Lützlower See	91.3	106	3.7	21.4	27.8	152	30.0	9.0	0.8	0.2

Age dependent encrustation

Encrustation was analysed in parts of plant thallus (Figure 17 and Appendix Table A3). A pattern of lowest in the first to third, middle in the forth to sixth, and highest carbonate content in the seventh to ninth whorls and internodes was found for both species (*C. canescens* and *C. hispida*) from Angersdorfer Teiche (Fisher LSD post-hoc test, $p < 0.01$). In Asche, Bruchwiesen, and Krüselinsee youngest part of *C. hispida*, *C. subspinosa*, and *C. tomentosa* was less encrusted than middle and oldest part of the plant thalli (Fisher LSD post-hoc test, $p < 0.001$). In Lützlower See, *C. subspinosa* and *C. tomentosa* exhibited no significant differences in encrustation between their respective parts of the thalli.

Further, encrustation was species-specific for species in Angersdorfer Teiche. *C. hispida* encrusted higher than *C. canescens*. Site-specific encrustation was found for *C. hispida* growing in Angersdorfer Teiche and Bruchwiesen with lower carbonate proportion of *C. hispida* in Bruchwiesen (Fisher LSD post-hoc test, $p < 0.01$). Also *C. tomentosa* encrusted site-specific in Asche compared to individuals from Krüselinsee and Lützlower See. Encrustation

of *C. tomentosa* was lower in Asche than in Krüselinsee and Lützlower See (Fisher LSD post-hoc test, $p < 0.001$).

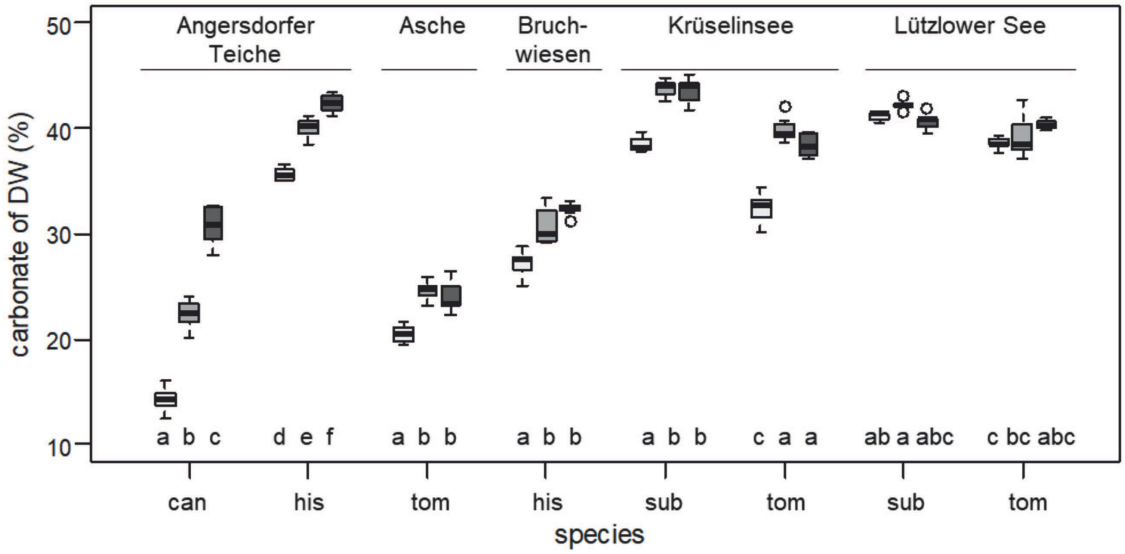

Figure 17: Comparison of carbonate content based on DW (%) of *C. canescens* (can), *C. hispida* (his), *C. subspinosa* (sub), and *C. tomentosa* (tom) from different sampling sites (Angersdorfer Teiche, Asche, Bruchwiesen, Krüselinsee, and Lützlower See). Plants were analysed in first to third (white), forth to sixth (light grey), seventh to ninth (dark grey boxplots) whorls and internodes. Box plots include whiskers (5-95 % of variability) and outliers (points). Different small letters indicate significant difference within the same sampling site (Tukey HSD post-hoc test, $p < 0.05$).

Plant element content

Element content based on plant dry weight was analysed for species which occurred in Angersdorfer Teiche, Asche, and Bruchwiesen (Figure 18 and Table 12). In the PCA plot, *C. hispida* from Bruchwiesen was separated from the species of Angersdorfer Teiche and Asche. Habitat differences were pronounced by means of lowest contents of Mg and Na in *C. hispida* from Bruchwiesen (Table 12). Further, parts of the thallus were ordered along the axis 1 which was characterised most by Ca, P, and Na contents.

Table 12: Comparison of plant element content (mean ± SD) of *C. canescens* (can), *C. hispida* (his), and *C. tomentosa* (tom) from Angersdorfer Teiche, Asche, and Bruchwiesen. Ca, K, Na, Mg, and P contents are presented as g kg^{-1} DW; K/Na mass ratios are calculated from weight. Different letters indicate significant differences (Tukey HSD post-hoc test, $p < 0.05$) in element content. Sampling size was n = 8.

site	species	Ca	K	Mg	Na	P	K/Na
Angersdorfer	can	94 ± 34.4 c	16.4 ± 1.7 a	12.6 ± 0.8 a	26.0 ± 2.3 a	1.6 ± 0.4 a	0.6 ± 0.1 d
Teiche	his	251.3 ± 41.8 a	11.2 ± 3.5 b	10.7 ± 0.7 b	14.7 ± 4.2 b	0.9 ± 0.4 b	0.8 ± 0.1 c
Asche	tom	158 ± 35.8 b	11.7 ± 3.6 b	11.7 ± 0.7 a	8.4 ± 2.3 c	0.9 ± 0.4 b	1.4 ± 0.1 b
Bruchwiesen	his	215.4 ± 17.3 a	14.7 ± 2.2 ab	6.3 ± 0.6 c	2.9 ± 1.0 d	1.0 ± 0.3 ab	5.5 ±1.4 a

For the element content, Ca was lowest in *C. canescens* and highest in *C. hispida* both from Angersdorfer Teiche. K content was significantly higher in *C. canescens* compared to *C. hispida* (Angersdorfer Teiche) and *C. tomentosa*. Na content was different for all species investigated; highest in *C. canescens* and lowest in *C. hispida* (Bruchwiesen). The ratio of K/Na was low (< 1) for both species from Angersdorfer Teiche and highest in *C. hispida* from Bruchwiesen. P content was significantly higher in *C. canescens* compared to *C. hispida* (Angersdorfer Teiche) and *C. tomentosa*.

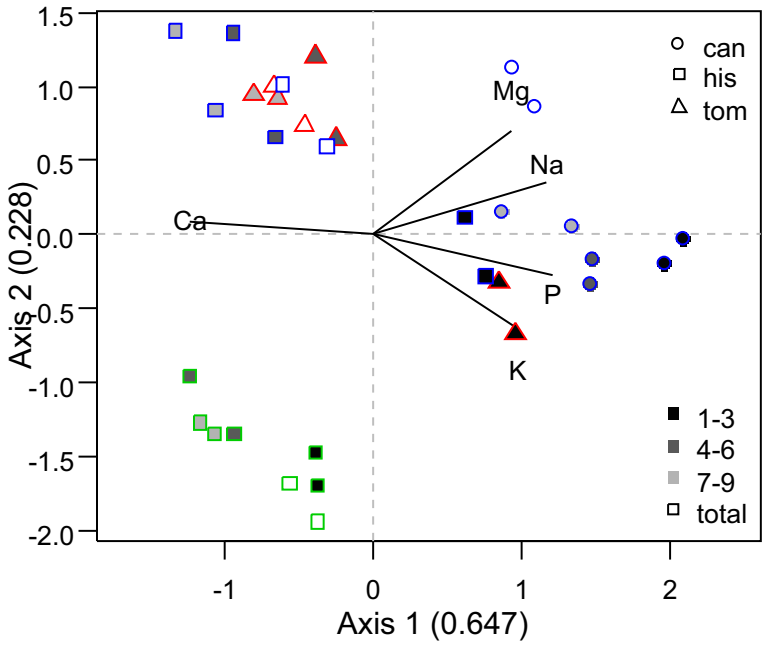

Figure 18: PCA plot of element contents (Ca, K, Mg, Na, and P) of charophytes from Angersdorfer Teiche (blue), Asche (red), and Bruchwiesen (green). Species were plotted with different symbols; background of symbols indicates parts of thallus, see legend.

4.4 Freshwater and brackish water habitats

Encrustation was studied of freshwater and brackish water specimens belonging to the same species. *C. aspera* and *C. tomentosa* from both freshwater (FW) and brackish water (BW) sites were investigated. For comparison, other charophyte species occurring in the same sites (*C. subspinosa* and *Nitellopsis obtusa*) were also collected.

Water chemistry

FW and BW sites were significant distinct with respect to water chemistry (Table 14 and Appendix Table A4). BW sites were characterised by higher concentrations of Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{+} and $SO_4{}^{2-}$, with Cl^{-} and Na^{+} concentrations exhibiting the most pronounced differences to FW. The concentration of TIC was slightly higher in FW. No significant differences between FW and BW were observed for TP concentration nor for pH, which was 8.3 in both groups of sites.

Encrustation

Precipitated carbonates, ash free dry weight, and mineral remains of plant dry weight were investigated by Henningsen (2017). Four samples for each of ten freshwater and six brackish water sites were analysed with the LOI method. Results of Henningsen (2017) are summarised in Figure 19 and Table 13.

The carbonate content was significantly higher in plants collected from FW than in BW plants (Kruskal-Wallis test, $p < 0.001$) (Figure 19 and Table 13).

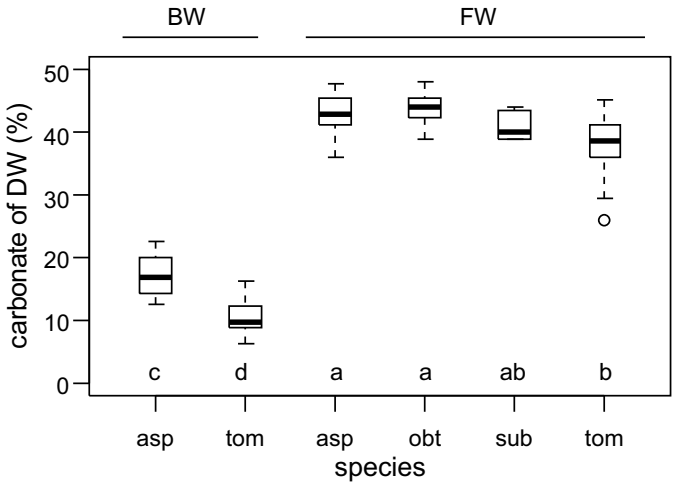

Figure 19: Comparison of carbonate content based on DW (%) of *C. aspera* (asp), *C. subspinosa* (sub), *C. tomentosa* (tom), and *N. obtusa* (obt) from FW and BW. Different letters indicate significant differences among groups (Fisher LSD post-hoc test, $p < 0.05$). Box plots include whiskers (5-95 % of variability) and outliers (points).

Within the BW sites, *C. aspera* had higher carbonate contents than *C. tomentosa* (Fisher LSD post-hoc test, $p < 0.001$). In FW, the differences in carbonate content were generally low, but contents were higher in *C. aspera* and *N. obtusa* than in *C. tomentosa* (Fisher LSD post-hoc test, $p < 0.001$).

Ash free dry weight was opposed to the proportion of carbonate. Plants from BW had higher contents of AFDW compared to plants from FW (Kruskal-Wallis test, $p < 0.001$). In general, the percentage of mineral remains was lower in plants from FW than in plants from BW sites (Kruskal-Wallis test, $p < 0.001$), while there were no significant differences in the content of mineral remains among species within FW or BW sites (Kruskal-Wallis test, $p \geq 0.05$). In FW the percentage of mineral remains of *C. aspera* and *C. tomentosa* were similar with 4.3 % ± 1.9 % of DW for *C. aspera* and 4.2 % ± 3.1 % of DW for *C. tomentosa*. In BW mineral remains of both species were higher than in FW (asp: 13.3 % ± 9.0 % of DW, tom: 7.7 % ± 3.9 % of DW).

Table 13: Comparison of carbonate, AFDW, and mineral remains proportion of DW from *C. aspera* (asp), *C. subspinosa* (sub), *C. tomentosa* (tom), and *N. obtusa* (obt). Charophytes were sampled in freshwater (FW) and brackish water (BW) sites. Different letters indicate significant differences (Fisher LSD post-hoc test, $p < 0.05$), n = number of sample size.

	species	n	carbonate	AFDW	mineral remains
FW	asp	16	42.8 ± 3.3a	24.3 ± 4.0c	4.3 ± 1.9 bc
	obt	8	43.9 ± 2.8a	24.6 ± 3.4c	2.2 ± 1.6c
	sub	12	40.8 ± 2.2ab	29.6 ± 3.9b	2.3 ± 1.0c
	tom	28	38.2 ± 4.3b	32.1 ± 6.0b	4.2 ± 3.1c
BW	asp	16	17.0 ± 3.3c	58.4 ± 6.5a	13.3 ± 9.0a
	tom	11	10.6 ± 2.8d	74.6 ± 2.6a	7.7 ± 3.9ab

Plant element content

Plant element content is shown in Table 15. Ca content based on dry weight was significantly higher for plants from FW sites compared to BW sites (Kruskal-Wallis test, $p < 0.001$). No difference of the Ca contents between *C. aspera* and *C. tomentosa* was found for FW sites. In BW sites, Ca contents of *C. aspera* were significantly higher than those in *C. tomentosa* (Fisher LSD post-hoc test, $p < 0.05$), which corresponds to the more pronounced encrustation of *C. aspera* in BW. If calculated based on the Ca contents measured here, the proportion of $CaCO_3$ in total encrustation differed between *C. aspera* and *C. tomentosa*. Figure 20 shows the relative proportion of $CaCO_3$ (calculated from Ca content) in total encrustation (as determined by loss of ignition) plotted against total encrustation per dry weight. For *C. tomentosa*, the proportion of $CaCO_3$ in encrustation rose with increasing encrustation (i. e. in freshwater), whereas the opposite pattern was observed for *C. aspera*.

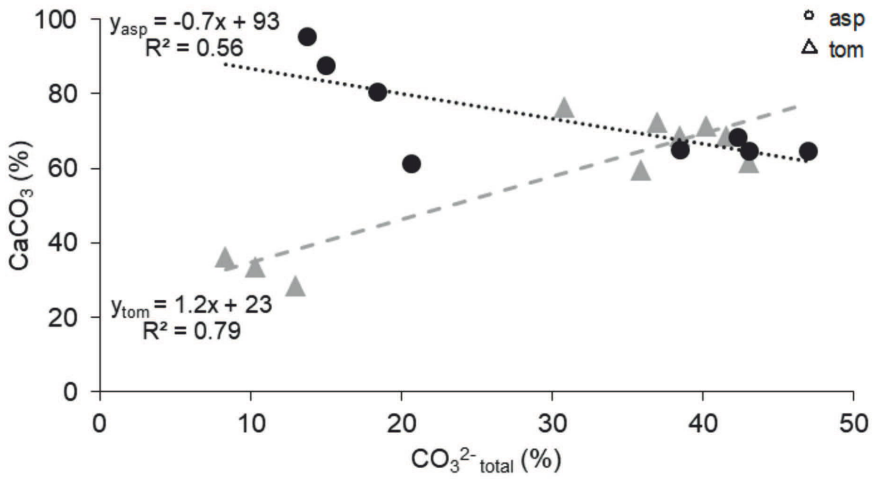

Figure 20: Dependency of the relative proportion of $CaCO_3$ (calculated from Ca-content) on total encrustation (CO_3 total, calculated from total encrustation per dry weight).

Species-specific differences were found for the K contents (Fisher LSD post-hoc test, $p < 0.001$). Neither for *C. aspera* nor *C. tomentosa*, K contents differed between FW and BW, but K contents of *C. aspera* were significantly lower than those in *N. obtusa*, *C. subspinosa* and *C. tomentosa*. *Nitellopsis obtusa* exhibited the highest K contents of all investigated species. Na contents exhibited both habitat-specific and species-specific differences. Na contents were significantly lower in plants from FW than from BW (Kruskal-Wallis test, $p < 0.001$). In FW, the lowest Na contents were found in *C. aspera* and *C. subspinosa*, followed by *C. tomentosa* and *N. obtusa*. In BW, Na contents in *C. tomentosa* were far higher than of *C. aspera*. The K/Na-ratios were higher for individuals collected from FW than from BW (Kruskal-Wallis test, $p < 0.001$). In BW, *C. aspera* had higher K/Na-ratios than *C. tomentosa* (Fisher LSD post-hoc test, $p < 0.05$). Mg contents were higher in plants collected from BW than in plants from FW sites (Kruskal-Wallis test, $p < 0.001$). Species-specific differences in Mg contents were found for brackish water conditions only, where *C. aspera* had lower Mg contents and Mg/Ca-ratios than *C. tomentosa*. In FW, TP contents of plant DW were significantly lower in *C. aspera* than in the other species. In contrast, *C. aspera* exhibited highest TP content in BW.

Interaction of investigated parameters

In the NMDS plot (dimensions = 2, stress = 0.03) plants from FW are distinctly separated from BW plants (Figure 21). FW plants form a single cluster, while BW plants are divided in two clusters, representing the two species investigated. Water chemistry parameter vectors reveal that the differentiation between FW and BW is dominated by conductivity, followed by TIC (permutation test, $p < 0.05$). The vectors of Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{+}, and $SO_4{}^{2-}$ concentrations of the sampling sites are not shown in Figure 21 for transparency reason, but they all are in line with conductivity vector.

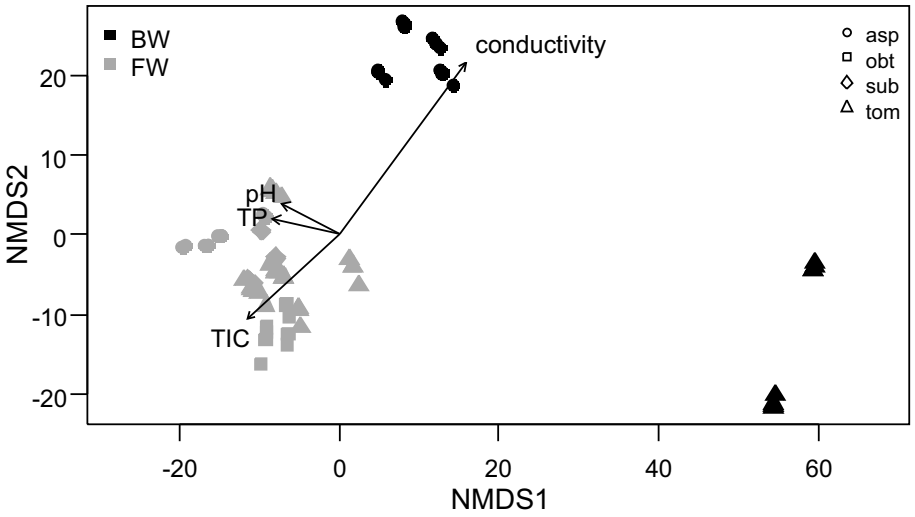

Figure 21: NMDS plot of element composition of Ca, K, Na, Mg, and P of the single species collected from brackish water (black) and freshwater (grey). Species are represented by different symbols, see legend. Water column parameters (TIC, TP, pH, and conductivity) were fitted as vectors in plot ($p < 0.05$).

Table 14: Comparison of water chemistry data of fresh- and brackish water habitats (mean ± SD). Listed are Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{+}, $SO_4{}^{2-}$, TIC and TP concentrations (mg L^{-1}), pH, and conductivity (mS cm^{-1}). Different letters indicate significant differences (Fisher LSD post-hoc test, $p < 0.05$) between fresh- (FW) and brackish water (BW) for the given parameter, n = number of sampling sites.

site	n	Ca^{2+}	Cl^{-}	K^{+}	Mg^{2+}	Na^{+}	$SO_4{}^{2-}$	TIC	TP	pH	cond
FW	10	49 ± 8.4 a	33 ± 24 a	3.3 ± 3.1 a	6.4 ± 4.4 a	18 ± 14 a	32 ± 20 a	28 ± 5.8 a	0.038 ± 0.03 a	8.3 ± 0.3 a	0.39 ± 0.08 a
BW	6	105 ± 15 b	3555 ± 638 b	86 ± 17 b	261 ± 44 b	2205 ± 356 b	520 ± 85 b	20 ± 2.4 b	0.026 ± 0.01 a	8.3 ± 0.4 a	11.6 ± 1.8 b

Table 15: Comparison of plant element content (mean ± SD) of Ca, K, Na, Mg and P of *C. aspera* (asp), *C. subspinosa* (sub), *C. tomentosa* (tom), and *N. obtusa* (obt) from FW and BW. Ca and Mg contents are presented as g kg^{-1} DW and K, Na, and P were calculated as g kg^{-1} AFDW. Mg/Ca and K/Na mass ratios are calculated from weight. Different letters indicate significant differences (Fisher LSD post-hoc test, $p < 0.05$) in element content of biomass, n = number of sample size.

	species	n	Ca	K	Na	Mg	P	Mg/Ca	K/Na
FW	asp	4	187 ± 15 a	12.3 ± 4.9 c	2.6 ± 0.4 c	1.4 ± 0.7 a	1.1 ± 0.5 d	0.01 ± 0.00 d	4.7 ± 1.5 b
	obt	2	186 ± 7.7 a	68.6 ± 5.6 a	8.8 ± 1.5 ab	2.7 ± 0.4 a	3.2 ± 0.9 ab	0.01 ± 0.00 c	8.0 ± 1.5 a
	sub	3	178 ± 9.9 a	31 ± 7.2 b	3.2 ± 1.1 c	3.1 ± 0.8 a	2.4 ± 0.5 bc	0.02 ± 0.00 c	10.1 ± 2.3 a
	tom	7	173 ± 17.7 a	45 ± 14.3 b	7.4 ± 3.4 b	2.9 ± 1.1 a	2.1 ± 0.8 c	0.02 ± 0.01 c	6.7 ± 4.7 b
BW	asp	4	89.7 ± 6.5 b	14.9 ± 3.5 c	7.3 ± 3.1 b	5.6 ± 0.8 b	3.9 ± 0.9 a	0.06 ± 0.01 b	2.3 ± 0.7 c
	tom	3	22.7 ± 2.3 c	34.1 ± 14.5 b	33.7 ± 12.7 a	6.9 ± 0.3 c	2.7 ± 0.6 b	0.31 ± 0.02 a	1.2 ± 0.0 d

4.5 Heavy metal exposure

The aim of this study was to assess the co-precipitation of lead, copper, cadmium, and zinc of the Schlüsselstollen water in the carbonate crust of charophytes and to determine if *Chara subspinosa* and *C. tomentosa* were found not only to withstand the lethal mix of heavy metals, but effectively to bioremediate them.

Water chemistry

Water chemistry of investigated sites is shown in Table A5 (Appendix). The adit Schlüsselstollen carried high concentrations of heavy metals (Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+}) into the Schlenze. Water of the Schlüsselstollen had a conductivity of 26.4 mS cm^{-1} which is diluted downriver. Above the Schlenze estuary ($Saale_{above}$) and in the Altarm concentrations of Cu^{2+} are detectable. 1.5 kilometres downstream of the Saale ($Saale_{below}$) Mg^{2+}, Na^{+}, Zn^{2+} concentrations, and conductivity were enhanced to $Saale_{above}$. In the Mansfelder region conductivity of sampling sites was higher referred to Lützlower See. Asche was characterised by high Ca^{2+} and Mg^{2+} concentrations. pH was lowest in Schlüsselstollen and in Asche whereas in all other study sites pH was ≥ 8.0.

Photosynthesis

The physiological state of the plants was measured by means of photosynthesis (Figure 22, 23, and Appendix Table A6). Electron transport rates (ETR_{max}) differed species-specific, higher in *C. tomentosa* than *C. subspinosa* (Fisher LSD post-hoc test, $p < 0.001$).

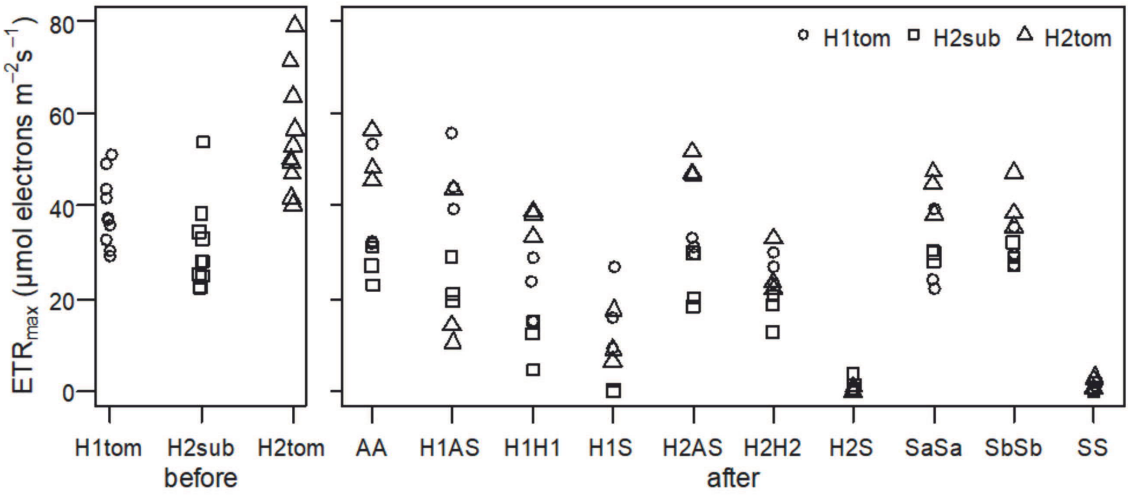

Figure 22: Electron transport rates (ETR_{max}) in µmol electrons $m^{-2}s^{-1}$ of plants before and after incubation. *C. subspinosa* (sub) and *C. tomentosa* (tom) from Asche (H1) and Lützlower See (H2) were planted in different sediment/water combinations: sediment and water from Asche (H1H1), Lützlower See (H2H2), $Saale_{above}$ (SaSa), $Saale_{below}$ (SbSb), Altarm (AA), Schlüsselstollen (SS). Sediment from Asche (H1) and from Lützlower See (H2) were also combined with Altarm:Schlüsselstollen (AS) and Schlüsselstollen (S) water. Species are presented as symbols, see legend.

When incubated in Schlüsselstollen water, plants had lowest electron transport rates (Fisher LSD post-hoc test, $p < 0.001$). Approaches with Schlüsselstollen water included different sediments from Asche, Lützlower See, and Schlüsselstollen (H1S, H2S, and SS). *C. subspinosa* did not withstand the heavy metal concentrations of Schlüsselstollen water in all types of sediments. However, *C. tomentosa* planted in sediment from Asche exhibited higher ETR_{max} compared to other sediments (Fisher LSD post-hoc test, $p < 0.05$). No significant difference in electron transport rates were found between all the other approaches (Fisher LSD post-hoc test, $p \geq 0.05$).

Fluorescence yield of dark-adapted plant ($Yield_{dark}$) was also lowest for species planted in water of the Schlüsselstollen (Figure 23 and Table A6). *C. tomentosa* planted in sediment of Asche exhibited higher fluorescence compared to *C. tomentosa* incubated in sediment of Lützlower See and Schlüsselstollen, all exposed to Schlüsselstollen water (Fisher LSD post-hoc test, $p < 0.05$).

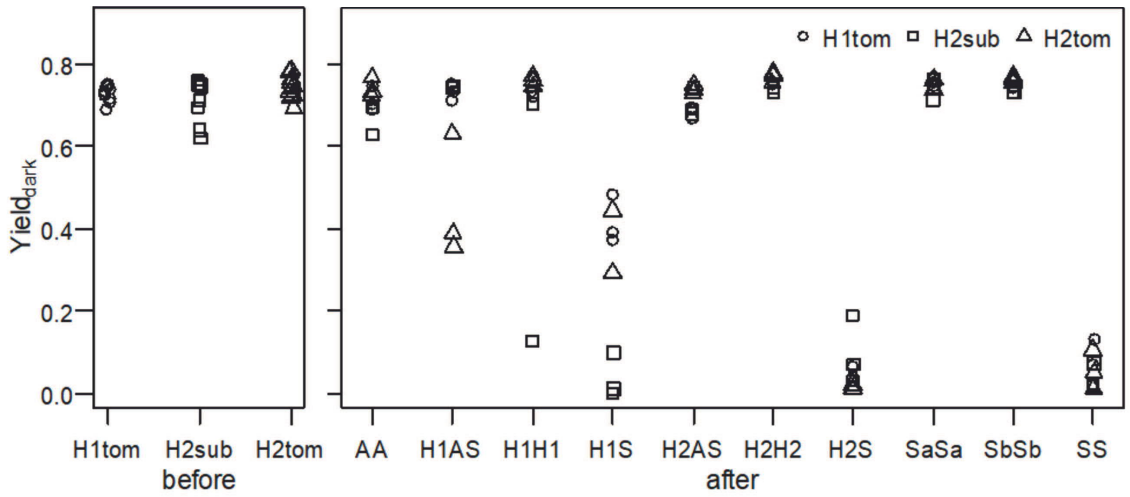

Figure 23: $Yield_{dark}$ of plants before and after incubation. *C. subspinosa* (sub) and *C. tomentosa* (tom) from Asche (H1) and Lützlower See (H2) were planted in different sediment/water combinations: sediment and water from Asche (H1H1), Lützlower See (H2H2), $Saale_{above}$ (SaSa), $Saale_{below}$ (SbSb), Altarm (AA), Schlüsselstollen (SS). Sediment from Asche (H1) and from Lützlower See (H2) were also combined with Altarm:Schlüsselstollen (AS) and Schlüsselstollen (S) water. Species are presented as symbols, see legend.

Photosynthetic pigments of chlorophyll *a*, *b*, and carotenoids were significantly lower when exposed to Schlüsselstollen water (Figure 24, 25, Appendix Table A6). Chlorophyll *a*/chlorophyll *b*-ratio (chl a/chl b) varied stronger of plants exposed to water of Schlüsselstollen. In these approaches chl a/chl b-ratio was lowest (Fisher LSD post-hoc test, $p < 0.05$). The ratio of chlorophyll *a*/carotenoid content was species-specific; lower in *C. tomentosa* (0.98 ± 0.58) than in *C. subspinosa* (4.63 ± 1.1). Chlorophyll *a*, *b* and carotenoid contents were significantly lower after heavy metals exposure in Schlüsselstollen water compared to the other approaches (Appendix Table A6).

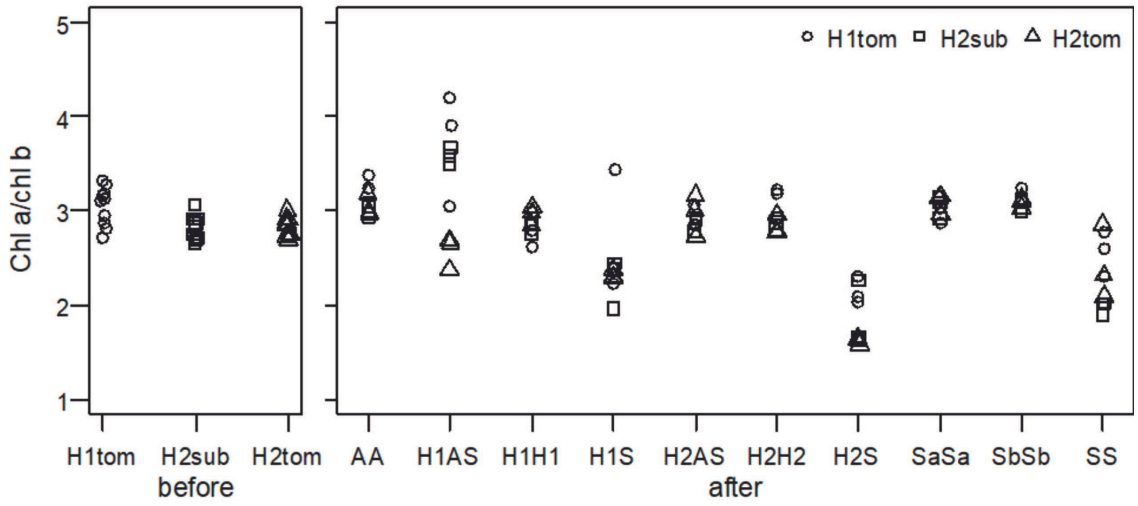

Figure 24: Chlorophyll *a*/chlorophyll *b*-ratio (chl a/chl b) of plants before and after incubation. *C. subspinosa* (sub) and *C. tomentosa* (tom) from Asche (H1) and Lützlower See (H2) were planted in different sediment/water combinations: sediment and water from Asche (H1H1), Lützlower See (H2H2), $Saale_{above}$ (SaSa), $Saale_{below}$ (SbSb), Altarm (AA), Schlüsselstollen (SS). Sediment from Asche (H1) and from Lützlower See (H2) were also combined with Altarm:Schlüsselstollen (AS) and Schlüsselstollen (S) water. Species are presented as symbols, see legend.

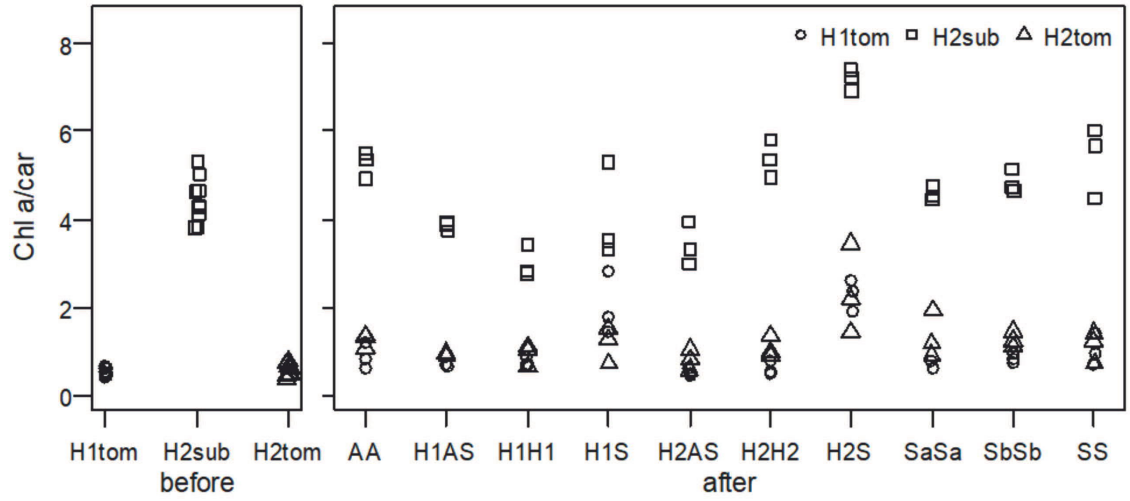

Figure 25: Chlorophyll *a*/carotenoid-ratio (chl a/car) of plants before and after incubation. *C. subspinosa* (sub) and *C. tomentosa* (tom) from Asche (H1) and Lützlower See (H2) were planted in different sediment/water combinations: sediment and water from Asche (H1H1), Lützlower See (H2H2), $Saale_{above}$ (SaSa), $Saale_{below}$ (SbSb), Altarm (AA), Schlüsselstollen (SS). Sediment from Asche (H1) and from Lützlower See (H2) were also combined with Altarm:Schlüsselstollen (AS) and Schlüsselstollen (S) water. Species are presented as symbols, see legend.

Absorption of heavy metals

Absorption of heavy metals was calculated from concentration difference before and after incubation of plants for ten days (Table 16). Initial concentration of heavy metals varied between investigated approaches (cf. Appendix Table A5). Negative values indicated resolved ions of sediments or plants. Highest absorption of Ca^{2+} was observed in approach with Asche sediment and Schlüsselstollen water (H1S). Ca^{2+} was resolved in approaches of sediment and water from Asche (H1H1) and sediment of Lützlower See and water of Schlüsselstollen (H2S). Cd^{2+} absorption was measureable in undiluted Schlüsselstollen water (H1S, H2S) but not in diluted Schlüsselstollen approaches (H1SA, H2SA) which had an initial Cd^{2+} concentration of 0.008 mg L^{-1}. For Cu^{2+}, absorption was significantly higher in undiluted than in diluted approaches with water of Schlüsselstollen (Turkey HSD post-hoc test, $p < 0.001$). Mg^{2+} concentration differed between approaches; absorption was positive for several approaches (H1H1, SaSa, AA, H2AS, H2S, and SS). Resolved concentration of Mg^{2+} was measured in approaches of H2H2, SbSb, H1AS, and H1S. Pb^{2+} concentration was only detected in water of Schlüsselstollen (H2S, and SS) but not in approaches with Asche sediment or diluted heavy metal concentrations. Absorption of Zn^{2+} was higher in diluted (H1AS, H2AS) than in undiluted Schlüsselstollen approaches (H2S, SS). Highest Zn^{2+} absorption was found in Asche sediment and Schlüsselstollen water (H1S).

Table 16: Difference of Ca^{2+}, Cd^{2+}, Cu^{2+}, Mg^{2+}, Pb^{2+}, and Zn^{2+} (mg L^{-1}) concentrations after incubation. Approaches are abbreviated with sediment and water from Asche (H1H1), Lützlower See (H2H2), $Saale_{above}$ (SaSa), $Saale_{below}$ (SbSb), Altarm (AA), Schlüsselstollen (SS). Sediment from Asche (H1) and Lützlower See (H2) were combined with water of Altarm:Schlüsselstollen (AS) and Schlüsselstollen (S). Different letter indicate significant difference (Tukey HSD or Fisher LSD post-hoc tests, $p < 0.05$). Sampling size was n = 6.

approach	Ca^{2+}	Cd^{2+}	Cu^{2+}	Mg^{2+}	Pb^{2+}	Zn^{2+}
H1H1	-5.1 ± 7.5 d			7.9 ± 6.3 a		
H2H2	28.8 ± 3.9 c			-0.25 ± 0.9 bcd		
SaSa	48.4 ± 4.0 a			0.22 ± 1.0 bcd		
SbSb	44.6 ± 7.1 ab			-0.21 ± 1.5 bcd		
AA	26.9 ± 5.2 c			3.6 ± 1.5 ab		
H1AS	27.1 ± 9.9 c		0.03 ± 0.00 a	-37.0 ± 4.6 d		2.5 ± 0.1 ab
H2AS	0.7 ± 3.6 c		0.02 ± 0.01 a	2.6 ± 1.4 abc		1.8 ± 0.1 bc
H1S	95.1 ± 21.0 a	0.02 ± 0.00 a	0.12 ± 0.01 b	-1.8 ± 4.9 cd		10.0 ± 0.4 a
H2S	-14.7 ± 16.3 d	0.01 ± 0.00 a	0.10 ± 0.03 b	3.2 ± 7.1 abc	0.13 ± 0.02 a	0.46 ± 1.1 c
SS	36.4 ± 8.0 bc		0.12 ± 0.02 b	13.0 ± 4.9 a	0.06 ± 0.04 b	-0.39 ± 2.2 c

Carbonate composition

Carbonate crust was analysed for element content of Ca, Cd, Cu, K, Mg, Mn, Na, P, Pb, and Zn (Table 17). Plant dry weight was composed of 19.5 % ± 6.0 % (SaSa) to 31.8 % ± 7.7 % (H1S) of carbonates which was similar between approaches. Highest element of the carbonate composition was Ca which differed significantly between addition of Saale, Altarm and Schlüsselstollen waters (SaSa, SbSb, AA and H2S, SS).

Table 17: Comparison of carbonate crust of plant DW (%) and its element content (mg g^{-1}) of Ca, K, Mg, Mn, Na, P, and co-precipitated Cd, Cu, and Zn. Sum of element contents (∑) is presented as % of carbonate crust. Approaches are abbreviated with sediment and water from Asche (H1H1), Lützlower See (H2H2), $Saale_{above}$ (SaSa), $Saale_{below}$ (SbSb), Altarm (AA), Schlüsselstollen (SS). Sediment from Asche (H1) and Lützlower See (H2) were combined with water of Altarm:Schlüsselstollen (AS) and Schlüsselstollen (S). Different letter indicate significant difference (Tukey HSD or Fisher's LSD post-hoc tests, $p < 0.05$), n = number of approach size.

approach	n	carboante crust	Ca	K	Mg	Mn	Na	P	∑
H1H1	6	27.3 ± 6.4 ab	144.1 ± 40.9 ab	32.3 ± 13.8 ab	17.9 ± 3.5 a	0.23 ± 0.22 ab	18.6 ± 6.6 bc	0.90 ± 0.23 cd	21.4 ± 1.9
H2H2	6	21. 3 ± 6.6 ab	137.6 ± 25.9 ab	31.9 ± 13.5 ab	8.9 ± 1.5 cde	0.04 ± 0.05 b	6.6 ± 4.4 d	0.62 ± 0.47 def	18.6 ± 3.2
SaSa	6	19.5 ± 6.0 b	160.7 ± 17.0 a	42.0 ± 6.7 ab	11.8 ± 1.8 abc	0.22 ± 0.41 ab	16.0 ± 2.6 cd	1.70 ± 0.55 abc	23.3 ± 1.0
SbSb	6	21.8 ± 5.9 ab	160.9 ± 28.9 a	47.9 ± 15.6 a	13.2 ± 2.4 ab	0.60 ± 0.12 a	15.7 ± 7.3 cd	2.25 ± 0.38 a	24.1 ± 1.6
AA	6	21.8 ± 4.8 ab	165.5 ± 28.5 a	40.9 ± 11.2 ab	13.5 ± 3.7 abc	0.28 ± 0.18 ab	14.2 ± 6.1 cd	2.26 ± 0.79 ab	23.7 ± 2.9
H1AS	6	25.5 ± 5.7 ab	138.7 ± 27.6 ab	26.1 ± 8.0 ab	10.3 ± 1.8 bcd	0.16 ± 0.17 ab	37.6 ± 11.8 ab	1.04 ± 0.37 bcd	21.4 ± 1.1
H2AS	6	28.6 ± 4.1 ab	127.6 ± 44.6 ab	23.9 ± 7.1 bc	7.6 ± 1.9 def	0.23 ± 0.10 ab	45.0 ± 17.0 a	0.86 ± 0.43 de	20.6 ± 2.6
H1S	6	31.8 ± 7.7 a	123.1 ± 32.6 ab	3.0 ± 1.1 d	7.9 ± 1.4 def	0.20 ± 0.16 ab	108.2 ±15.8 a	0.28 ± 0.17 ef	24.3 ± 1.4
H2S	6	30.9 ± 5.3 ab	93.4 ± 49.5 b	3.1 ± 0.7 cd	5.7 ± 0.8 ef	0.10 ± 0.08 b	108.8 ± 29.6 a	0.14 ± 0.04 f	21.2 ± 2.6
SS	6	30.3 ± 6.5 ab	77.8 ± 36.3 b	1.9 ± 0.6 d	4.8 ± 1.8 f	0.09 ± 0.09 b	98.1 ± 34.3 a	0.10 ± 0.06 f	18.3 ± 6.3

approach	Cd	Cu	Zn
H1H1			
H2H2		0.005 ± 0.002 c	
SaSa		0.008 ± 0.001 bc	0.026 ± 0.029 c
SbSb	0.008 ± 0.001 a	0.01 ± 0.001 b	0.019 ± 0.011 c
AA		0.031 ± 0.009 a	0.030 ± 0.019 bc
H1AS			0.064 ± 0.047 bc
H2AS	0.005 ± 0.001 a	0.005 ± 0.001 c	0.656 ± 0.310 a
H1S			0.301 ± 0.186 ab
H2S	0.002 ± 0.000 b	0.027 ± 0.013 a	0.588 ± 0.251 a
SS	0.003 ± 0.001 b	0.023 ± 0.010 ab	0.618 ± 0.328 a

Cd was co-precipitated in the carbonate crust; higher in water of $Saale_{below}$ (SbSb) than in Schlüsselstollen water (H2S and SS), which exhibited highest Cd concentrations at the beginning of the experiment. For Cd and Cu, elements were not co-precipitated when incubated with sediment from Asche (H1H1, H1AS, and H1S). In all other approaches Cu was detected in the carbonate formation of exposed plants. K, Mg, and P contents differed between approaches and were lowest in the precipitated carbonates with water of Schlüsselstollen. Mn co-precipitated in the carbonate crust of all investigated approaches. Na content depended on concentration; highest co-precipitation was found incubated in water with highest Na^+ concentration. Pb was not detectable in the carbonate composition of all incubated plants. Zn co-precipitated highest in approaches with heavy metal solution of Schlüsselstollen (diluted and undiluted). Furthermore, Zn was detected in the encrustation of plants exposed to water of Saale (above and below) and Altarm water.

Sediments

Carbonate content of sediments was compared before and after incubation with different waters (Figure 26 and Appendix Table A7). Significant difference in carbonate contents were found in sediments of Asche and Lützlower See both exposed to Schlüsselstollen water (H1S, H2S) (Fisher LSD post-hoc test, $p < 0.001$). Sediments of Lützlower See (H2H2) and $Saale_{below}$ (SbSb) which were incubated with their habitat water differed in carbonate contents (Fisher LSD post-hoc test, $p < 0.01$). Carbonate contents of other sediments were similar before and after incubation (Fisher LSD post-hoc test, $p \geq 0.01$).

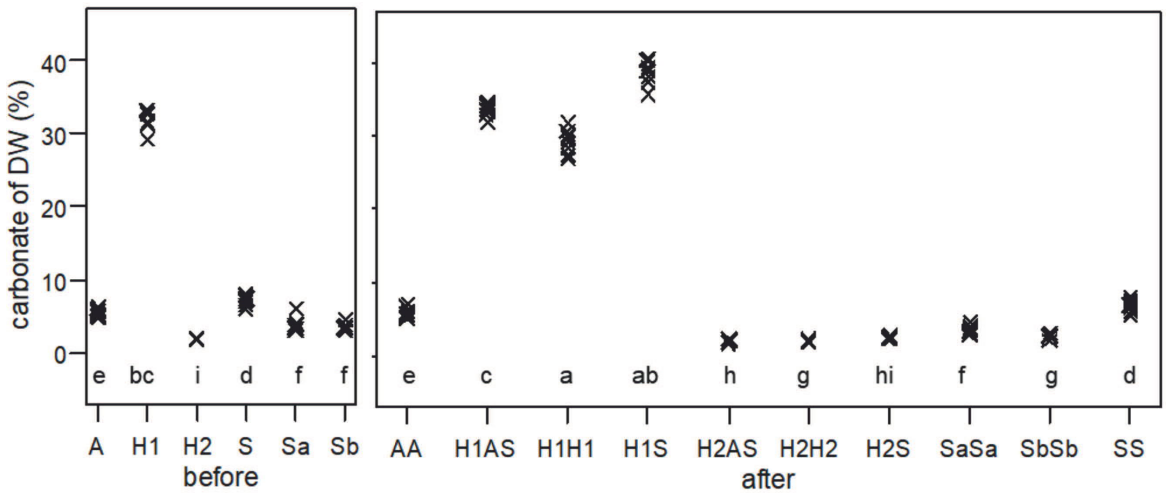

Figure 26: Comparison of sediments carbonates content. Sediments before incubation are abbreviated as Altarm (A), Asche (H1), Lützlower See (H2), Schlüsselstollen (S), $Saale_{above}$ (Sa), and $Saale_{below}$ (Sb). After incubation sediment and water from Asche (H1H1), Lützlower See (H2H2), $Saale_{above}$ (SaSa), $Saale_{below}$ (SbSb), Altarm (AA), Schlüsselstollen (SS) are listed. Sediment from Asche (H1) and from Lützlower See (H2) were also combined with Altarm:Schlüsselstollen (AS) and Schlüsselstollen (S) water. Different letters indicate significant difference (Fisher LSD post-hoc test, $p < 0.05$).

5 Discussion

The investigations presented here focused on the identification of factors significant for the encrustation of charophytes. Assuming that encrustation of charophytes is a process related to growth, three topics were investigated in detail: seasonal pattern, species specificity and habitat characteristics, which will be discussed below.

5.1 Seasonal pattern

$CaCO_3$ precipitation and element content of charophytes from Krüselinsee and Lützlower See were investigated monthly for a period of one year. Water chemistry parameters of the two hard-water lakes were also analysed. The resulting seasonal patterns are discussed here.

$CaCO_3$ precipitation of plant dry weight was species-specific for all species that occurred in Krüselinsee or Lützlower See, which corresponds to findings of several other authors (Kufel et al. 2013, 2016; Pukacz et al. 2016b). In Krüselinsee, the highest carbonate content of investigated plants was found. Both species, the corticated *C. contraria* and the ecorticated *N. obtusa*, precipitated the highest carbonate content of DW in June and August. In March, *C. subspinosa* precipitated the lowest carbonate content in Krüselinsee. In this context, *N. obtusa* exhibited a higher carbonate content than *C. subspinosa* for all months sampled, except for April. By contrast, Kufel et al. (2016) found higher carbonate contents in *C. subspinosa* than in *N. obtusa* in five lakes investigated. Similarly, *C. tomentosa* encrusted less than *C. globularis* in Lützlower See, whereas Pukacz et al. (2016b) found higher encrustation of *C. tomentosa* than *C. globularis* in three lakes studied. The comparisons confirm that habitat-specificity, especially water ion composition, is decisive for species-specific encrustation in hard-water lakes which is discussed later.

In Lützlower See, *N. flexilis/opaca* (ecorticated) precipitated the lowest $CaCO_3$ content compared to *C. tomentosa* and *C. globularis* (both corticated). In connection with the above mentioned encrustation of charophytes from Krüselinsee, it can be concluded that cortication is not determining the degree of encrustation, at least in calcareous hard-water lakes.

Although Ca^{2+} concentration of Krüselinsee (36.9 ± 5.5 mg L^{-1}) was lower than that in Lützlower See (95.0 ± 3.6 mg L^{-1}), the highest species-specific encrustation was observed in Krüselinsee. However, *C. subspinosa* and *C. tomentosa* growing in both lakes exhibited higher encrustation in Lützlower See than in Krüselinsee. The exception is the carbonate precipitation of *C. subspinosa* and *C. tomentosa* in July and August where encrustation was similar. It can therefore be concluded that Ca^{2+} concentration, because of its large difference between the two lakes, is not the only determinant for the encrustation of charophytes.

Furthermore, the correlation between the precipitated $CaCO_3$ and the Ca^{2+} concentration in the water body was negative for both lakes during the course of the year. A similar negative correlation for *C. tomentosa* was already reported by Pukacz et al. (2016b). This negative correlation indicates that the seasonality of growth, rather than the Ca^{2+} concentration, is decisive for the encrustation of characean plants in the lakes studied. Consequently, the total vegetation in the lake could impact on the Ca^{2+} concentration in the water due to $CaCO_3$ precipitation. During vegetation season, Ca^{2+} and DIC concentrations were consumed and were lowest at maximal characean growth, an observation made by Pentecost et al. (2006). Utilization of bicarbonate could affect the carbon cycle of lakes, a topic discussed by Kufel and Kufel (2002) and Pukacz et al. (2014b).

For TIC, a strong and significant negative correlation to $CaCO_3$ precipitation was found, which corresponds to the C-uptake model presented by Pedersen et al. (2013), as well as the laboratory experiments by Wang et al. (2013). Wang et al. (2013) also showed in laboratory experiments that encrustation is affected by dissolved inorganic carbon under hard-water conditions. On a species level, negative correlations of TIC and precipitated $CaCO_3$ were found for all species (except for *C. subspinosa* at Lützlower See and *C. tomentosa* at Krüselinsee). Because TIC exhibited a clear seasonality as well as encrustation, this observed pattern can be regarded as another example of seasonality of precipitated carbonates as already reported by Pukacz et al. (2014b, 2016a). The general pattern described by these authors is in agreement with the data presented; lowest encrustation in April and highest in July and August.

This seasonal pattern could be explained by the connection of encrustation and photosynthesis by an active process of Ca^{2+} extrusion in alkaline zones, triggered by an antiport of H^+ and Ca^{2+} in the regions where CO_2-uptake takes place (McConnaughey and Falk 1991; Brownlee and Taylor 2002; Pedersen et al. 2013). C-uptake was found to be species-specific for *C. corallina*, *C. globularis*, and *C. fibrosa* sampled at the same depths (Sorrell et al. 2001). It supports species-specific C-uptake for photosynthesis but does not further explain the species-specific encrustation, because the two processes are directly linked to each other. Consequently, the encrustation should be related primarily to the biomass production, resulting in almost constant carbonate to dry weight ratios across all species. The species-specific differences observed in this study must be explained by mechanisms also relying on proton transport, but not balanced by Ca^{2+} extrusion, for instance nutrient uptake (McConnaughey and Whelan 1997; Ullrich et al. 1998).

No seasonal pattern for *C. subspinosa* in Lützlower See and *C. tomentosa* in Krüselinsee was found. It must be noted that *C. subspinosa* and *C. tomentosa* did exhibit a seasonal pattern of encrustation, and a negative correlation between $CaCO_3$ precipitation and TIC, at the respective other lake. The reason for the missing correlation therefore is lacking seasonality

of encrustation at the respective lake for the given species, which points to site-specificity. For both species, the pattern of significant correlations to the water chemistry parameters clearly proves the habitat-specificity of the encrustation. The data confirms habitat-specific encrustation of charophytes, an observation also described by Coletta et al. (2001), Kufel et al. (2013, 2016), and Pukacz et al. (2016b). Consequently, seasonality of encrustation can be regarded as a phenomenon observed for all species investigated, but expressed in a site-specific manner.

Encrustation corresponds to Ca content of plant dry weight which was also species-specific. The highest Ca content was found in *C. contraria* (Krüselinsee) and the lowest in *N. flexilis*/*opaca* (Lützlower See), irrespective of higher Ca^{2+} concentration in Lützlower See. This result is in contrast to Mg, where content was almost constant for all the species within a lake, but differed significantly between the lakes. Both elements can thus be regarded as tightly controlled and kept stable, either independent (Ca^{2+}) or dependent on the external conditions (Mg^{2+}). A different pattern was found for the Fe contents, where the habitat-specific differences were negligible, but *C. globularis* and *N. flexilis*/*opaca* exhibited higher values compared to the other species. This effect should be investigated in more detail, because all the species, except for *C. tomentosa* in Lützlower See, showed significant correlations between Fe content and the $CaCO_3$ precipitation of plant dry weight.

K content in plant dry weight showed a clear species-specificity; irrespective of K^+ concentration differences in the lakes, *C. subspinosa* and *C. tomentosa* had the same K contents in Krüselinsee and Lützlower See, respectively. Also, the seasonality parameter was less pronounced for the internal K content, showing a significant relationship to the $CaCO_3$ precipitation only for *C. subspinosa* and *C. globularis*. The large distribution of the species-specific contents, as well as the observed short-term variability (differences between sampling dates without seasonal pattern) of the biomass K content, concurs with the central role of K for the regulation of the transport processes, as well as for the stabilisation of turgor (Spear et al. 1969; Beilby 1986; Shimmen and MacRobbie 1987; Winter and Kirst 1990).

Co-precipitation of PO_4^{3-} with the encrustation is discussed by several authors (Otsuki and Wetzel 1972; Murphy et al. 1983; Kufel and Kufel 2002). Kufel et al. (2016) observed the highest P content of DW for *C. globularis* and *C. intermedia*, while encrustation was the lowest in both species. The results are consistent with the negative correlation of precipitated $CaCO_3$ and P content of plant DW investigated in this study.

The NMDS plot (Figure 14) summarised the element composition of investigated charophytes and confirmed that TIC and seasonality are the most important parameters in determining the content of Ca, Fe, K, Mg, and P. The seasonal differences in the element content could be caused by the development of the plant itself. Winter et al. (1987) proved seasonal changes in ionic concentration of the vascular sap which depended on age and developmental

state of *C. vulgaris*. The impact of the developmental state on element composition and encrustation of charophytes is discussed in the following paragraphs.

To this end, encrustation and element composition was studied in two charophyte species (*C. subspinosa* and *C. tomentosa*) prior to and during formation of gametangia. *C. tomentosa* develop oogonia and antheridia on different individuals (dioecious) while *C. subspinosa* is monoecious (Migula 1897; Krause 1997). In this process, the relation of photosynthesis to biomass production is shifted towards accumulation of reserve substances (Remmert 1978).

For *Chara vulgaris*, Winter et al. (1987) described reduced growth and highest sucrose concentration during maturation. The ionic content was decreased to maintain the osmotic pressure (Winter et al. 1987). Therefore, it is assumed that element content differs in charophytes whilst fertile. Accumulation of reserve substances should be highest in female plants followed by monoecious, and lowest in male and non-fertile plants. As photosynthesis is linked to encrustation, at least in hard-water lakes (Smith 1967; McConnaughey 1991), fertility could indirectly affect the precipitation of carbonates.

Difference in encrustation between fertile and non-fertile plants was found for dioecious (*C. tomentosa*) but not for monoecious plants of *C. subspinosa* (monoecious). Fertile plants encrusted higher in whorls of *C. subspinosa* and female *C. tomentosa* compared to internodes. No difference in precipitated carbonates was detected in whorls and internodes of male C. tomentosa. Differences in element content were found for P in fertile/non-fertile and whorl/internode of *C. tomentosa*. Hence, the following assumption is made: the link between photosynthesis (CO_2 uptake) and encrustation ($CaCO_3$ precipitation), as well as the negative correlation of precipitated $CaCO_3$ and P content of plants investigated, allows for the comparison of $CaCO_3$/P as C/P-ratio. In June, the $CaCO_3$/P-ratio was lower for fertile compared to non-fertile *C. tomentosa*. Both female and male whorls of C. tomentosa had the lowest ratio of $CaCO_3$/P in July. This finding indicates enhanced carbohydrates content, e. g. as reserve substances which are accumulated during fertility (Winter and Kirst 1991b, 1992; Kaźmierczak 2001). However, further research is needed to confirm this result.

It can be concluded that both seasonality, as well as habitat-specific water chemistry parameters, determine the encrustation of charophytes in hard-water lakes. For both seasonality as well as habitat-specificity, a pronounced species-specificity was observed which confirm the first two hypotheses tested in this study. As a consequence, temporal resolution of the species-specific and the habitat-specific encrustation must be respected, at least in hard-water habitats. Sampling must take into consideration the phenology of the species, as well as the habitat-specific variability of water chemistry parameters. However, it should be noted that for extrapolation of encrustation data to other habitats, more knowledge about species-specific dependency from water chemistry parameters is necessary. The following investigations are therefore conducted in habitats with distinct water chemistry.

5.2 Habitat specificity

Habitat-specific encrustation and element content pattern of charophytes from freshwater and brackish water individuals were compared. A distinct habitat-specific difference was observed in the encrustations of plants from freshwater and brackish water. Individuals from brackish water were less encrusted than individuals from freshwater. For the first time, the results present quantitative data for observations made a long time ago (Hasslow, 1931; Wahlstedt, 1875). As the mean pH value was 8.3 in both FW and BW, these differences in encrustation cannot be explained by different pH conditions of the habitats. Charophytes are known for their ability to alter pH, at least close to the cell surface (Spear et al. 1969; Beilby and Casanova 2014). These effects could not be measured directly in this study, but within a given habitat all factors affecting the alteration of surface pH (buffer capacity) will act similarly for the species compared.

Schütte (2003) observed higher ash content in brackish water than in freshwater of *C. aspera* and assumed that the ash content of freshwater plants mainly consisted of carbonate encrustations which is in line with the results of this study. To make a comparison, carbonate contents of freshwater and brackish water sites were multiplied by 1.67 (fraction of CO_3^{2-} in $CaCO_3$) to achieve the calcium carbonate content. Both for BW and FW, the absolute values of calcium carbonate content correspond to already published data. For brackish water, Urbaniak (2010) reported ash contents for *C. baltica* Bruzelius 1824 (26.8 % ± 1.7 % of DW) comparable to *C. aspera* (28.3 % ± 5.6 % of DW) in this study, whereas *C. tomentosa* (17.7 % ± 4.7 % of DW) from the same site exhibited lower encrustation. For freshwater, published ranges of calcium carbonate encrustation are 70 % to 78 % for *C. aspera* (Schütte 2003); 50 % to 69 % for *C. subspinosa* (Pukacz et al. 2014b; Kufel et al. 2016); 60 % to 71 % for *C. tomentosa* (Blindow 1992b; Kufel et al. 2016; Pukacz et al. 2016b) and 55 % to 71 % for *N. obtusa* (Urbaniak 2010; Kufel et al. 2013, 2016).

Chara aspera was more encrusted than *C. tomentosa*. Due to more distinct differences in encrustations of *C. tomentosa* between FW and BW, the difference between the species was even more pronounced in brackish water. The lower salinity tolerance of *C. tomentosa* and differences in the mechanisms of turgor regulation could possibly explain this difference. The upper salinity limit of *C. tomentosa* is 7.5 psu (Torn et al. 2003) whereas *C. aspera* occurs at salinities up to 18 psu (Nielsen 2003). *Chara aspera* collected from brackish water sites is well adapted to 10 psu (Blindow et al. 2003). As shown by Winter and Kirst (1992), *C. aspera* actively regulates K^+, Na^+, Cl^-, and sucrose concentrations resulting in perfect turgor stability up to 8 psu and allows for increasingly moderate growth up to 18 psu. Other halotolerant species such as *C. tomentosa* and *N. obtusa* regulate their turgor by Na^+ and Cl^-, but keep K^+ concentrations constant (Winter and Kirst 1990, 1991b; Winter et al. 1999). Consequently, the K^+/Na^+-ratios drop and reach lethal values below 1 at a salinity

of 5-7 psu (Winter and Kirst 1990). In fact, the K/Na-ratios in the plants collected from brackish water sites were above 2 for *C. aspera*, but only 1.2 for *C. tomentosa* (Table 15). Following the argumentation of Winter et al. (1999), such a decreased K/Na-ratio indicates approaching the physiological limit, which is in agreement with the upper salinity limit of 7 psu for *C. tomentosa*.

K content in plants showed a pronounced species-specificity, but no difference between BW and FW for the two species occurring in both habitats, which is in strong agreement with the results of Bisson and Kirst (1995). Both *C. aspera* and *C. tomentosa* have limited osmoregulation and regulate the turgor without increasing K contents within the investigated salinity ranges. The Na content in the plant biomass was both habitat- and species-specific. The fact that *N. obtusa* and *C. tomentosa* did not show differences in Na content corresponds with the findings of Winter and Kirst (1990) and Winter et al. (1999). The authors indicated identical mechanisms of turgor regulation for these two species. Similarly, the strong increase in Na contents of *C. tomentosa* at BW conditions compared to the only moderate increase of *C. aspera* correspond with the different turgor regulation mechanisms outlined above.

Both *C. aspera* and *C. tomentosa* had lower P contents in FW than in BW. Correlation of P content and encrustation was negative (Spearman correlation test, $r = -0.72$, $p < 0.01$) which indicates that only a minor part of the plant phosphorus is bound in the carbonate formation. The results support earlier assumptions that P is mainly stored in the organic part of the plants (Siong and Asaeda 2009; Kufel et al. 2013).

Surprisingly, there is a large gap between the carbonate losses determined by LOI and the potential calcium carbonate content calculated from plant element analysis. Even if assuming that not only all Ca content, but also all Mg is bound in carbonates, about 30 % of the freshwater carbonates are not assigned to calcium or magnesium. For *C. tomentosa* the gap is most pronounced in brackish water, where about 60 % of the carbonates obtained by LOI are not bound to Ca and Mg contents of the plants. As both potassium and sodium carbonates are far more soluble than calcium and magnesium carbonates, the plant contents of the former two ions are unlikely to explain the gap. Instead, it is assumed that potassium origin from the organic plant fractions, where K is an important contributor to the highly species-specific turgor regulation (Bisson and Kirst 1995). The lack of any correlation between K and carbonate contents of plant dry weight supports this assumption. However, the Na content was significantly increased in *C. tomentosa* compared to *C. aspera* from BW (Table 15), which could explain the species-specific difference of the gap but does not elucidate the remaining difference. Precipitation of other metal carbonates (e. g. Sr, Mn), as demonstrated to occur in charophytes by several authors (McConnaughey 1991; Anadon et al. 2002; Schöler et al. 2014) can serve as a hypothesis for further investigations of this effect.

In this context, the causes of the surprising species differences in encrustation and element content patterns should be investigated further. Mg contents of both species were far higher in brackish sites than in freshwater sites. Within the brackish water sites, *C. tomentosa* had far higher Mg contents than *C. aspera*, but lower Ca and carbonate contents (the latter calculated from LOI). Species-specific trends of the proportion of $CaCO_3$ in total encrustation (calculated from Ca content) were observed. *Chara tomentosa* had a lower proportion of $CaCO_3$ in BW than in FW, whereas *C. aspera* had a higher proportion of $CaCO_3$ in BW. This indicates major differences in the mechanisms determining the amount of encrustation between the two species and is in strong agreement with the observations of Anadon et al. (2002).

It has been reported that high concentrations of Mg^{2+} inhibit encrustation of charophytes (Siong and Asaeda 2009; Gomes and Asaeda 2010; Asaeda et al. 2014). In the brackish water sites investigated here, Mg^{2+} concentrations were far higher than in freshwater and even exceeded Ca^{2+} concentrations; a condition that suppresses $CaCO_3$-formation (Rushdi et al. 1992; Chen et al. 2005). This pattern has been explained by absorption of Mg^{2+} on the surface of calcium carbonate, which changes the characteristics of surface roughness and crystal distortion (Chen et al. 2005). Akin and Lagerwerff (1965) described increased solubility of calcium carbonate at high $SO_4{}^{2-}$ concentrations. The far higher $SO_4{}^{2-}$ concentrations in brackish water compared to freshwater could alternatively explain the much lower encrustations in brackish water, which have been observed for charophytes in this study and by other researchers.

The amounts of encrustation and related parameters (Ca and Mg contents) differed significantly between freshwater and brackish water. These differences can mainly be explained by physicochemical differences of habitats (ion composition), creating an increased demand for osmoregulation and consequently resulting in altered activity of ion channels (Beilby et al. 2018). This general picture is modified by species-specific physiological peculiarities of osmoregulation, as differences between *C. aspera* and *C. tomentosa* in brackish water were found. The K contents, the major ion involved in turgor regulation, showed a strong species-specificity but no habitat-specificity, while content of Na differed both between habitats and among species.

Furthermore, encrustation was studied in brackish waters with strong ion anomalies. Increased ion concentrations were found in Angersdorfer Teiche, Asche and Bruchwiesen. For comparison, samples were also taken in two hard-waters lakes; Krüselinsee and Lützlower See. Carbonate precipitation was highest for charophytes in hard-water lakes and of *C. hispida* in Angersdorfer Teiche. This was not expected for *C. hispida* growing in Angersdorfer Teiche at a salinity of 6.3. As shown above, encrustation of charophytes decreased from FW to BW. Thus, ion composition rather than ion concentration is decisive for encrustation

of charophytes. It could further explain the low carbonate precipitation of *C. tomentosa* in Asche, where Mg^{2+} exceeded Ca^{2+} concentrations, and consequently inhibits the encrustation as seen for charophytes in BW sites. Also, high $SO_4{}^{2-}$ concentrations in Asche and Bruchwiesen could reduce the encrustation of charophytes, as described by Akin and Lagerwerff (1965).

The results emphasise the importance of water ion composition on carbonate precipitation of charophytes, which was strengthened regarding the site-specific pattern of encrustation along the age gradient of the plants. Here 'age' referred to the cell age along the plant thallus at a certain state. Site-specific patterns of encrustation were found: all parts of plant thalli (youngest, middle, and oldest) were different; the youngest differed from the other parts, and encrustation was similar for all parts of the thalli. Kawahata et al. (2013) confirmed age dependent encrustation along internodes of *C. globularis*. Younger internodes were less encrusted than older ones (Kawahata et al. 2013). Carbonate formations were also detected between the cortex and cell wall which indicated that encrustation was enclosed during forming of the cortex (Kawahata et al. 2013). Three levels of carbonate formation can therefore be distinguished; external, intra-thallus carbonates precipitation and internal calcification of oospores which are termed gyrogonites (Kawahata et al. 2013; Raven et al. 1986; Leitch 1991).

The age pattern of encrustation in this study showed a strong site-specificity, whereas encrustation of charophytes was species-specific. In Angersdorfer Teiche, the most pronounced difference in encrustation between species (*C. hispida* and *C. canescens*) and cell age (youngest, middle, and oldest part) was detected. The strong difference in species-specific encrustation could be explained by its low K/Na contents, which was 0.8 for *C. canescens* and 0.6 for *C. hispida* (Table 12). Winter and Kirst (1990) reported reduced vitality of charophytes at a ratio of $K^+/Na^+ < 1$, but found also ratios of 0.8-0.9 in *Tolypella glomerata* Leonh. 1863 and *T. nidifica* Braun 1856 (Winter et al. 1996) when exposed to salinity of 12 psu. Consequently both *C. hispida* as well as *C. canescens*, were most probably growing close to their physiological limits in Angersdorf.

In summary, it was shown that encrustation of charophytes from inland brackish water sites with strong ion anomalies could be as high as in freshwater. It is assumed that ion composition, rather than concentration of the water, impact on the encrustation of charophytes.

In the context of the research done, charophytes were exposed to water with increased ion and heavy metal concentrations. Co-precipitation of heavy metals by charophytes was studied for its bioremediation potential. To estimate the extent of this potential, Schlüsselstollen water with enhanced heavy metal concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+} was used.

Photosynthesis, measured by means of ETR_{max} and $Yield_{dark}$, were lowest for *Chara subspinosa* and *C. tomentosa* cultivated in Schlüsselstollen water compared to diluted approaches. This indicates toxicity on both electron transports and integrity of photosystem II. In addition, chlorophyll *a*, *b* and carotenoid contents were also reduced due to the toxic effect of the Schlüsselstollen water. Reduction in chlorophyll pigments of charophytes were also found by Sooksawat et al. (2013) when exposed to Cd^{2+} (0.5 mg L^{-1}) and Pb^{2+} (10 mg L^{-1}). Exposure to 10 mg L^{-1} Zn^{2+} did not significantly influence the chlorophyll *a*, *b* and carotenoid contents in charophytes (Sooksawat et al. 2013). Besides the heavy metal concentrations in Schlüsselstollen water, conductivity was enhanced (26.4 mS cm^{-1}). However, plants withstand the Schlüsselstollen water when diluted with water of Altarm.

The uptake capacity of Cd, Cu, Pb, and Zn in *Chara subspinosa* was analysed of single and multi metal addition by Patzelt (2017). Plants were cultivated on a daily addition of Cd^{2+} (0.04 mg L^{-1}), Cu^{2+} (0.03 mg L^{-1}), Pb^{2+} (0.5, 1 mg L^{-1}), Zn^{2+} (2 mg L^{-1}), and 10 % Schlüsselstollen water (Patzelt 2017). The addition of ion concentrations differed due to different concentrations in the Schlüsselstollen water. Absorption of individual metals varied; cadmium and zinc were retained in much higher concentrations than copper and lead. In Schlüsselstollen water, heavy metals absorption was lower, except of Cd^{2+}, which showed that an uptake limit was reached (Patzelt 2017). In comparison, absorption of heavy metals in this study gave a rough indication because ion concentrations were only measured before and after the cultivation of 10 days and not on a daily basis as Patzelt (2017).

Accumulation of Cd, Cu, Pb, and Zn in charophytes was analysed in the plant DW by several authors (Siong and Asaeda 2009; Bibi et al. 2010; Gao and Yan 2012; Sooksawat et al. 2013; Laffont-Schwob et al. 2015). In this study, co-precipitation of heavy metals in the carbonate crust and not in the plant dry weight was investigated. Charophytes precipitated Cd (0.002 to 0.008 mg g^{-1}), Cu (0.002 to 0.031 mg g^{-1}) and Zn (0.018 to 0.656 mg g^{-1}) in the carbonate crust, which were lower compared to the accumulations in charophyte DW presented by other authors (Table 18).

Table 18: Comparison of Cd, Pb, and Zn accumulation in charophyte dry weight (mg g^{-1}). Species were exposed to different concentrations of Cd^{2+}, Pb^{2+}, and Zn^{2+} (mg L^{-1}) for a certain incubation time (days).

species	element	content (mg g^{-1} DW)	medium conc. (mg L^{-1})	incubation time	reference
C. fibrosa	Cd	1.3	0.01	21	Siong and Asaeda 2009
N. graciliformis	Cd, Zn	0.3, 2.5	0.15, 1	35	Bibi et al. 2010
C. globularis	Pb	3.7	160	15	Gao and Yan 2012
N. opaca	Cd, Pb	1.5, 21.7	0.5, 10	6	Sooksawat et al. 2013
C. aculeolata	Zn	6.5	10	6	Sooksawat et al. 2013

Laffont-Schwob et al. (2015) investigated Cd, Cu, Pb, and Zn content of *Chara* spp. in temporary ponds close to a highway. All metals were accumulated in *Chara* spp., but only in low contents; 4 µg Cd, 517 µg Cu, 177 µg Pb, and 1800 µg Zn in kg^{-1} DW (Laffont-Schwob et al. 2015). Compared to the investigation of Laffont-Schwob et al. (2015), Cd, Cu and Zn were co-precipitated higher by charophytes in this study. However, Pb was not detected in the carbonate crust of charophytes exposed to Schlüsselstollen water.

Although Pb was not detected in the carbonate encrustation, Pb^{2+} concentration was reduced or no longer detectable in the water of the experimental approaches. It was therefore assumed that lead was quickly removed from the water column, probably by sedimentation. The assumption made corresponds to the findings of Gao and Yan (2012), who found higher Pb depositions in soil surface than in charophyte dry weight at all investigated concentrations (Gao and Yan 2012). Furthermore, changes of dissolved solids and metal concentrations of the Schlüsselstollen water were studied by Baborowski and Bozau (2006) and Baborowski and von Tümpling (2012). Due to its high conductivity, Schlüsselstollen water sinks when discharged into the Schlenze (Baborowski and Bozau 2006). The Schlenze flows into the Saale where they are mixed and thus increases the particular proportion of elements (Baborowski and von Tümpling 2012). Especially for Pb, this effect was highest when tested in laboratory experiments (Baborowski and von Tümpling 2012).

Furthermore, carbonate content of sediments were analysed before and after the experiment. Difference in carbonate contents were found in sediments of Lützlower See (before: 1.9 % ± 0.1 %, after: 2.1 % ± 0.1 % of DW) and $Saale_{below}$ (before: 3.5 % ± 0.5 %, after: 2.7 % ± 0.3 % of DW) which was low (Lützlower See) or less after cultivation ($Saale_{below}$). However, further differences in carbonate contents of sediment from Lützlower See (before: 1.9 % ± 0.1 %, after: 2.5 % ± 0.2 % of DW) and Asche (before: 32.3 % ± 0.5 %, after: 38.9 % ± 1.6 % of DW) cultivated with Schlüsselstollen water were increased. The latter exhibited the highest increase of carbonate content after exposure to heavy metals. Plants cultivated in sediment of Asche and water of Schlüsselstollen (H1AS and H1S) did not precipitate Cd and Cu in the carbonate crust. Also Zn precipitation was lower compared to plants exposed to the same heavy metal concentration but planted in a different type of sediment. In addition, ETR_{max} and $Yield_{dark}$ were significantly higher in *C. tomentosa* planted in sediment of Asche when exposed to Schlüsselstollen water. All of this indicated, that at least Pb was sedimented (e. g. as lead carbonate) but also Cd^{2+} and Zn^{2+} concentration could be reduced from the water (Baborowski and von Tümpling 2012), especially when cultivated with Asche sediment.

Based on the achieved results, bioremediation efficiency of a charophyte meadow was calculated. To this end, charophyte biomass data of Pukacz et al. (2014a) was used. Meadows of 100 % charophyte covering exhibited an average biomass of 1165 g m^{-2} in Lake Jasne (Pukacz et al. 2014a). Calculated with the average biomass of Pukacz et al. (2014a) and the average carbonate crust (25.7 % ± 7.1 %) of investigated plants, DW resulted in 299 g carbonate per m^{-2} biomass (Table 19). The highest and lowest element contents of Cd, Cu, and Zn were used to identify the range of co-precipitated elements in a charophyte meadow. 0.6-2.4 mg Cd, 0.6-9.3 mg Cu, and 5.4-196.4 mg Zn could co-precipitate in a charophyte meadow (m^{-2}). Co-precipitation of elements with the lowest and highest biomass production was also calculated.

Table 19: Calculation of element content of Cd, Cu, and Zn in carbonate layer of charophyte biomass (m^{-2}). Charophyte dry weight (g m^{-2}, first line) of Pukacz et al. (2014a) was used to calculate the carbonate bound elements (mg m^{-2}).

	minimum	maximum	mean
dry weight (g m^{-2})	417	2067	1165
carbonate (g m^{-2})	107.2	531.2	229.4
Cd (mg m^{-2})	0.2 – 0.9	1.1 – 4.2	0.6 – 2.4
Cu (mg m^{-2})	0.2 – 3.3	1.1 – 16.5	0.6 – 9.3
Zn (mg m^{-2})	1.9 – 70.3	9.6 – 348.5	5.4 – 196.4

Finally, the composition of the carbonate crust of investigated plants is discussed. The proportion of carbonate crust was no different between heavy metal exposed and non-exposed plants. On the contrary, Gomes and Asaeda (2013) showed decreased encrustation (30 % – 40 %) of charophytes when exposed to Cd and Cr. Exposed plants had further reduced Ca contents (Gomes and Asaeda 2013). In this study, Ca content of the carbonate composition was also lower for charophytes planted in Schlüsselstollen water. The same pattern was found for K, Mg, and P contents, which were lowest in the carbonate composition of plants cultivated with Schlüsselstollen water. For Mg, a higher content was found for charophytes cultivated in Asche water which correspond to the highest Mg^{2+} concentration of sites investigated. A strong impact on the Na content of the carbonate composition was found; Na content correlated with the Na^{+} concentration of the respective water (Figure 27). In Schlüsselstollen water, Na content was as high as Ca content of the carbonate composition. Therefore, it is assumed that Na is deposited on the carbonate structure and inhibits precipitation of other elements (Ca, K, Mg, and P).

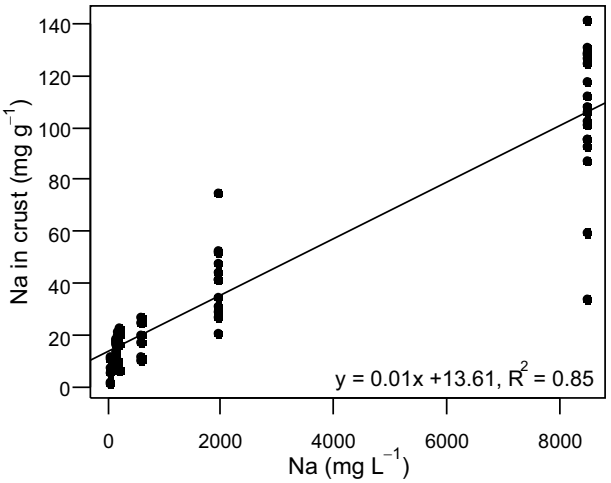

Figure 27: Dependency of Na content in the carbonate crust (mg g^{-1}) on Na^+ concentration (mg L^{-1}) of the surrounding water.

Summarising, charophytes withstand the toxic heavy metal concentrations of Schlüsselstollen water, when it was diluted with water of Altarm. Plants encrusted similarly in different approaches. Co-precipitation of Cd, Cu, and Zn in the carbonate crust of plants was shown. Pb was not detected in the carbonate crust and was probably deposited in sediments. However, physico-chemical characteristics of waters with high ion concentrations (e. g. Schlüsselstollen) interact with the carbonate composition of charophytes. Na content in the carbonate crust depended on the Na^+ concentration of the surrounding water. Carbonate composition of charophytes (Ca > K > Mg > Na > P > Mn) was shifted towards Na content when cultivated in Schlüsselstollen water. In brackish waters with a strong ion anomaly, ion composition rather than ion concentration, and here especially the Na^+-ratio to other ions, is decisive for the encrustation of charophytes. The results confirm the hypothesis that habitat-specificity influence on encrustation and composition of carbonate crust of charophytes.

5.3 Future research

This investigation showed that in addition to water chemistry, developmental state including seasonality impact on the encrustation of charophytes.

Three aspects, resulting from the study are of special interest. The first, and most intriguing one, is the role Na^+ plays in encrustation. It was shown that Na content of the crusts increased far above equilibrium values with increasing Na^+ concentrations of the water. This implies an active accumulation of Na^+ in the crusts which, with respect to the high solubility of Na carbonates, remains to be explained. Two hypothesis to be further investigated can be deduced from this: i) Na^+ is extruded actively from the cells and accumulated within the diffusion barrier when a solid crust is forming; ii) solubility of complex carbonates, where Na^+ is just a component, are much lower than of pure Na carbonate.

A second aspect resolved by this study was the striking difference in encrustation of charophytes growing in marine-brackish water bodies and brackish water with strong ion anomalies. Whereas encrustation decreased with increasing salinity, no such differences were observed in water bodies of comparable osmotic values, but strong ion anomaly. Again, two hypotheses can be deduced: i) osmoregulation (with turgor regulation as the main component) cannot be seen as the main or only mechanism decreasing encrustation by enhanced demand for proton pumping or, alternatively, ii) the requirement for osmoregulation is dependent on ion composition, shifting the limits for the individual osmoregulatory mechanisms according to ion composition. In this context, the difference between Ca content and LOI determined carbonate content should be kept in mind for forthcoming publications. LOI is determining carbonate loss, which is not *per se* identical with calcium compounds.

The third and last aspect is an applied one. At least some species exhibited, as expected, a potential for bioremediation of heavy metals. However, Na^+ concentration and sediment composition influenced this capacity in an unexpected degree. For efficient bioremediation, which is mainly targeting heavy metals, a two-step process should be tested; where first Na^+ is removed and then, in a second pond, heavy metals are removed from the Na^+ depleted water. In the example studied here, the potential for installation of a low-cost (with respect to both installation as well as maintenance) bioremediation plant was demonstrated. Diluted with river water, charophytes were accumulating heavy metals from the polluted Schlüsselstollen outlet. A two-step plant, where first Na^+ concentration is lowered and in a second step, heavy metals are removed, should be tested in order to solve the recent pollution problem of the Saale/Elbe river system.

An observation requiring further attention is the lack of phosphate co-precipitation. Further studies targeting specifically the phosphate content of crusts in relation to water chemistry are required in order to identify the conditions under which such co-precipitation does or does

not occur. As a result of all the data currently available originating from hard-water lakes and sometimes even dealing with the same species, ion composition details and/or phosphate levels are the most likely candidates to explain the discrepancies between the results of the different authors.

References

Akin, G W and Lagerwerff, J V (1965). Calcium carbonate equilibria in aqueous solutions opent to the to the air. I. The solubility of calcite in relation to ionic strength. *Geochimica Et Cosmochimica Acta* 29, 343–352.

Anadon, P, Utrilla, R, and Vazquez, A (2002). Mineralogy and Sr-Mg geochemistry of charophyte carbonates: a new tool for paleolimnological research. *Earth and Planetary Science Letters* 197, 205–214.

Apolinarska, K, Pełechatyb, M, and Pukaczc, A (2011). $CaCO_3$ sedimentation by modern charophytes (Characeae): can calcified remains and carbonate $\delta\ ^{13}C$ and $\delta\ ^{18}O$ record the ecological state of lakes? – a review. *Studia Limnologica et Telmatologica*, 55–66.

Asada, K (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology* 141, 391–396.

Asaeda, T, Senavirathna, M D H J, Kaneko, Y, and Rashid, M H (2014). Effect of calcium and magnesium on the growth and calcite encrustation of *Chara fibrosa*. *Aquatic Botany*, 100–106.

Atapaththu, K S S, Rashid, M H, and Asaeda, T (2016). Growth and oxidative stress of brittlewort (*Nitella pseudoflabellata*) in response to cesium exposure. *Bulletin of Environmental Contamination and Toxicology* 96, 347–353.

Baborowski, M and Bozau, E (2006). Impact of former mining activities on the uranium distribution in the River Saale (Germany). *Applied Geochemistry* 21, 1073–1082.

Baborowski, M, Mages, M, Hiltscher, C, Matschullat, J, and Guhr, H (2005). Former mining activities influence Uranium concentrations in the Elbe river near Magdeburg. In: Uranium in the environment. Mining impact and consequences. Ed. by B J Merkel and A Hasche-Berger. Springer, Berlin, 585–592.

Baborowski, M and von Tümpling, W (2012). Umsetzung Sedimentmanagementkonzept Schwermetalleinträge Schlüsselstollen in die Saale. Abschlussbericht: Laufzeit Juli 2012 - 14.12.2012. *Helmholtz-Zentrum für Umweltforschung – UFZ, Department Fließgewässerökologie*, 1–41.

Beilby, M (1986). Factors controlling the K^+ conductance in *Chara*. *The Journal of Membrane Biology* 93, 187–193.

Beilby, M J and Bisson, M A (2012). pH banding in charophyte algae. In: Plant Electrophysiology, Methods and Cell Electrophysiology. Ed. by Volkov A. Springer, Berlin, 247–271.

Beilby, M J and Casanova, M T (2014). The physiology of characean cells. Springer, Berlin, 1–205.

Beilby, M J, Cherry, C A, and Shepherd, V A (1999). Dual turgor regulation response to hypotonic stress in *Lamprothamnium papulosum*. *Plant, Cell and Environment* 22, 347–359.

Beilby, M J, Shepherd, V A, and Absolonova, M (2018). The role of H^+/OH^- channels in saline pathology of *Chara australis*: brief history. *Botany Letters* 165, 45–54.

Bibi, M H, Asaeda, T, and Azim, E (2010). Effects of Cd, Cr, and Zn on growth and metal accumulation in an aquatic macrophyte, *Nitella graciliformis*. *Chemistry and Ecology* 26, 49–56.

Bicudo, C E de M and Bueno, N C (2013). Characeae biomass: is the subject exhausted? In: Biomass Now - Sustainable Growth and Use. Ed. by M D Matovic. IntechOpen, 524–540.

Bisson, M A and Bartholomew, D (1984). Osmoregulation or turgor regulation in *Chara*? *Plant physiology* 74, 252–255.

Bisson, M A and Kirst, G O (1995). Osmotic acclimation and turgor pressure regulation in algae. *Naturwissenschaften* 82, 461–471.

Blindow, I (1992a). Decline of charophytes during eutrophication: comparison with angiosperms. *Freshwater Biology* 28, 9–14.

Blindow, I (1992b). Long- and short-term dynamics of submerged macrophytes in two shallow eutrophic lakes. *Freshwater Biology* 28, 15–27.

Blindow, I (2000). Distribution of charophytes along the Swedish coast in relation to salinity and eutrophication. *International Review of Hydrobiology* 85, 707–717.

Blindow, I, Dietrich, J, Mollmann, N, and Schubert, H (2003). Growth, photosynthesis and fertility of *Chara aspera* under different light and salinity conditions. *Aquatic Botany* 76, 213–234.

Blindow, I, Hargeby, A, and Andersson, G (2002). Seasonal changes of mechanisms maintaining clear water in a shallow lake with abundant *Chara* vegetation. *Aquatic Botany* 72, 315–334.

Blindow, I, Hargeby, A, and Hilt, S (2014). Facilitation of clear-water conditions in shallow lakes by macrophytes: differences between charophyte and angiosperm dominance. *Hydrobiologia* 737, 99–110.

Blindow, I and van de Weyer, K (2016). Ökologie der Characeen. In: Armleuchteralgen - Die Characeen Deutschlands. Ed. by AG Characeen Deutschlands. Springer, Berlin, 79–95.

Brownlee, C and Taylor, A R (2002). Algal calcification and silification. *Encyclopedia of Life Sciences*, 1–6.

Bulychev, A A, Cherkashin, A A, Rubin, A B, Vredenberg, W J, Zykov, S V, and Müller, S C (2001). Comparative study on photosynthetic activity of chloroplasts in acid and alkaline zones of *Chara corallina*. *Bioelectrochemistry* 53, 225–232.

Casanova, M T and Brock, M A (1990). Charophyte germination and establishment from the seed bank of an Australian temporary lake. *Aquatic Botany* 36, 247–254.

Chen, T, Neville, A, and Yuan, M (2005). Influence of Mg^{2+} on $CaCO_3$ formation – bulk precipitation and surface deposition. *Journal of Crystal Growth* 275, 1341–1347.

Clabeaux, B L, Navarro, D A, Aga, D S, and Bisson, M A (2013). Combined effects of cadmium and zinc on growth, tolerance, and metal accumulation in *Chara australis* and enhanced phytoextraction using EDTA. *Ecotoxicology and Environmental Safety* 98, 236–243.

Claus, E, Kasimir, P, John, H J, Möhlenkamp, C, Becker, B, Hillebrand, G, and Heininger, P (2015). Untersuchung von Sedimenten in ausgewählten Staustufen, Nebenflüssen und Seitenstrukturen im Unterlauf der Saale. *Hydrologie und Wasserbewirtschaftung* 59, 305–317.

Coletta, P, Pentecosta, A, and Spiro, B (2001). Stable isotopes in charophyte incrustations: relationships with climate and water chemistry. *Palaeogeography Palaeoclimatology Palaeoecology* 173, 9–19.

de Mendiburu, F (2016). agricolae: Statistical Procedures for Agricultural Research. R package version 1.2-4.

Doege, A, van de Weyer, K, Becker, R, and Schubert, H (2016). Bioindikation mit Characeen. In: Armleuchteralgen - Die Characeen Deutschlands. Ed. by AG Characeen Deutschlands. Springer, Berlin, 79–138.

Eilers, P H C and Peeters, J C H (1988). A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecological Modelling* 42, 199–215.

Fernández-Aláez, M, Fernández-Aláez, C, and Rodriguez, S (2002). Seasonal changes in biomass of charophytes in shallow lakes in the northwest of Spain. *Aquatic Botany* 72, 335–348.

FGG ELBE (2015). Weniger strenge Bewirtschaftungsziele für die im deutschen Teil der Flussgebietseinheiten Elbe und Oder durch den Braunkohlenbergbau und den Sanierungsbergbau beeinflussten Grundwasserkörper. *Geschäftsstelle der Flussgebietsgemeinschaft Elbe*, 1–104.

FGG ELBE Anlage 7 (2015). Anhang A4-5: Umweltqualitätsnormen zur Beurteilung des chemischen Zustands (Anlage 7 der OGewV). *Geschäftsstelle der Flussgebietsgemeinschaft Elbe*, 1–3.

Fox, J and Weisberg, S (2011). An R Companion to Applied Regression. Second. Sage, Thousand Oaks.

Friese, K, Hupfer, M, and Schultze, M (1998). Chemical characteristics of water and sediment in acid mining lakes of the Lusatian lignite district. In: Acidic Mining Lakes. Environmental Science. Ed. by W Geller, H Klapper, and W Salomons. Springer, Berlin.

Fritsch, F E (1935). The structure and reproduction of the algae. *Cambridge University Press, Cambridge*, 447–469.

Gao, Y and Yan, X (2012). Response of *Chara globularis* and *Hydrodictyon reticulatum* to lead pollution: their survival, bioaccumulation, and defense. *Journal of Applied Phycology* 24, 245–251.

García, A (1994). Charophyta: their use in paleolimnology. *Journal of Paleolimnology* 10, 43–52.

Gomes, P I A and Asaeda, T (2009). Phycoremediation of chromium (VI) by *Nitella* and impact of calcium encrustation. *Journal of Hazardous Materilas*, 1332–1338.

Gomes, P I A and Asaeda, T (2010). Impact of calcium and magnesium on growth and morphological acclimations of *Nitella*: implications for calcification and nutrient dynamics. 26, 479–491.

Gomes, P I A and Asaeda, T (2013). Phytoremediation of heavy metals by calcifying macroalgae (*Nitella pseudoflabellata*): implications of redox insensitive end products. *Chemosphere* 92, 1328–1334.

Gosek, A, Kwiatkowska, M, and R, Duszyński (1996). The effect of cadmium on growth of vegetative thallus and development of generative organs of *Chara vulgaris* L. after short time of cultivation. *Acta Societatis Botanicorum Poloniae*, 67–72.

Graham, L E, Cook, M E, and Busse, J S (2000). The origin of plants: body plan changes contributing to a major evolutionary radiation. *Proceedings of the National Academy of Sciences* 97, 4535–4540.

Guiry, M D and Guiry, G M (2018). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org, 24.06.2018.

Hargeby, A, Andersson, G, Blindow, I, and Johansson, S (1994). Trophic web structure in a shallow eutrophic lake during a dominance shift from phytoplankton to submerged macrophytes. *Hydrobiologia* 279, 83–90.

Hartwig, H J, Knitzschke, G, and Kuyumcu, M (1999). 800 Jahre Kupferschieferbergbau – Die Kupferschiefererzlagerstätten Mansfeld/Sangerhausen und ihre bergbauliche Nutzung 1200-1990. In: Kali-, Steinsalz und Kupferschiefer in Mitteldeutschland. Ed. by O Hartmann. GGW 205, Berlin, 23–35.

Hasslow, O J (1931). Sveriges characéer. *Botaniska Notiser*, 128–130.

Heiri, O, Lotter, A F, and Lemcke, G (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology* 25, 101–110.

Henningsen, L (2017). Kalkinkrustierung verschiedener Characeenarten in Süß- und Brackwasser, Master thesis. *University of Greifwald*, 1–69.

Heumann, H G (1987). Effects of heavy metals on growth and ultrastructure of *Chara vulgaris*. *Protoplasma* 136, 37–48.

Hoffmann, R and Bisson, M A (1990). *Chara buckeiii*, a euryhaline Charophyte from an unusual saline environment. *Plant Physiology* 93, 122–127.

Hutchinson, G E (1975). A Treatise on Limnolog (Limnological botany). Wiley, New York.

Jeffrey, C (1967). The origin and differentiation of the Archegoniate land plants: a second contribution. *Kew Bulletin* 21, 335–349.

Kairesalo, T, Gunnarsson, K, Jónsson, G S, and Jónasson, P M (1987). The occurrence and photosynthetic activity of epiphytes on the tips of *Nitella opaca* Ag. (Charophyceae). *Aquatic Botany* 28, 333–340.

Kalin, M, Wheeler, W N, and Meinrath, G (2005). The removal of uranium from mining waste water using algal/microbial biomass. *Journal of Environmental Radioactivity* 78, 151–177.

Kawahata, C, Yamamuro, M, and Shiraiwa, Y (2013). Changes in alkaline band formation and calcification of corticated charophyte *Chara globularis*. *SpringerPlus* 2, 1–6.

Kaźmierczak, A (2001). The relationships between fertility and contents of gibberellic acid, sugars and dry mass in apical parts of *Chara vulgaris* thalli. *Biologia Plantarum* 44, 439–441.

Kingsford, R T and Porter, J L (1994). Waterbirds on an adjacent freshwater lake and salt lake in arid Australia. *Biological Conservation* 69, 219–228.

Koroleff, F (1983). Simultaneous oxidation of nitrogen and phosphorus compounds by persulfate. In: Methods of Seawater Analysis. Ed. by K Grasshoff, K Kremling, and M Ehrhardt. Wiley, Weinheim, 168–169.

Krause, W (1981). Characeen als Bioindikatoren für den Gewässerzustand. *Limnologica* 13, 399–418.

Krause, W (1997). *Charales* (Charophycae). In: Süsswasserflora von Mitteleuropa, Band 18. Ed. by H Ettl, G Gärtner, H Heynig, and D Mollenhauer. Gustav Fischer, Jena, 168–169.

Kuczyńska-Kippen, N (2007). Habitat choice in rotifera communities of three shallow lakes: impact of macrophyte substratum and season. *Hydrobiologia* 593, 27–37.

Kufel, L, Biardzka, E, and Strzałek, M (2013). Calcium carbonate incrustation and phosphorus fractions in five charophyte species. *Aquatic Botany* 109, 54–57.

Kufel, L and Kufel, I (2002). *Chara* beds acting as nutrient sinks in shallow lakes – a review. *Aquatic Botany* 72, 249–260.

Kufel, L, Strzałek, M, and Biardzka, E (2016). Site- and species-specific contribution of charophytes to calcium and phosphorus cycling in lakes. *Hydrobiologia* 767, 185–195.

Küster, A, Schaible, R, and Schubert, H (2004). Light acclimation of photosynthesis in three charophyte species. *Aquatic Botany* 79, 111–204.

Lacerda, L D, Fernandez, M A, Calazans, C F, and Tanizaki, K F (1992). Bioavailability of heavy metals in sediments of two coastal lagoons in Rio de Janeiro, Brazil. *Hydrobiologia* 228, 65–70.

Laffont-Schwob, I, Triboit, F, Prudent, P, Soulié-Märsche, I, Rabier, J, Despréaux, M, and Thiéry, A (2015). Trace metal extraction and biomass production by spontaneous vegetation in temporary Mediterranean stormwater highway retention ponds: Freshwater macroalgae (*Chara* spp.) *vs*. cattails (*Typha* spp.) *Ecological Engineering* 81, 173–181.

LAF/PLEJADES (2013). 4011GVV: Frachtreduzierung Schlüsselstollen, Bericht zum Arbeitspaket A: Ermittlung der Auswirkungen des Schlüsselstollens auf den partikel-gebundenen Schadstofftransport in der Saale/Elbe, Magdeburg, 1–71.

Leitch, A R (1991). Calcification of the charophyte oosporangium. In: Calcareous Algae and Stromatolites. Ed. by R Riding. Springer, Berlin, 204–216.

Lucas, W J (1975). Photosynthetic fixation of ^{14}Carbon by internodal cells of *Chara corallina*. *Journal of Experimental Botany* 26, 331–346.

Lucas, W J, Keifer, D W, and Sanders, D (1983). Bicarbonate transport in *Chara corallina*: evidence for cotransport of HCO_3^- with H^+. *The Journal of Membrane Biology* 73, 263–274.

Lucas, W J and Smith, F A (1973). The formation of alkaline and acid regions at the surface of *Chara corallina* cells. *Journal of Experimental Botany* 24, 1–14.

Mages, M, von Tümpling, W, Veen, A van der, and Baborowski, M (2006). Element determination in natural biofilms of mine drainage water by total reflection X-ray fluorescence spectrometry. *Spectrochimica Acta - Part B Atomic Spectroscopy* 61, 1146–1152.

Marquardt, M and Schubert, H (2009). Photosynthetic characterisation of *Chara vulgaris* in bioremediation ponds. *Charophytes* 2, 1–8.

McConnaughey, T (1991). Calcification in *Chara corallina* carbon dioxide hydroxylation generates protons for bicarbonate assimilation. *Limnology and Oceanography* 36, 619–628.

McConnaughey, T A and Falk, R H (1991). Calcium-proton exchange during algal calcification. *Biological Bulletin* 180, 185–195.

McConnaughey, T A and Whelan, J F (1997). Calcification generates protons for nutrient and bicarbonate uptake. *Earth Science Reviews* 42, 95–117.

McCourt, R M (1995). Green algal phylogeny. *Trends in Ecology and Evolution* 10, 159–163.

Melzer, A (1994). Möglichkeiten einer Bioindikation durch submerse Makrophyten – Beispiele aus Bayern. *Beiträge zur angewandten Gewässerökologie Norddeutschlands* 1, 92–102.

Migula, W (1897). Die Characeen Deutschlands, Oesterreichs und der Schweiz. Ed. by Rabenhorst L. Kummer, Leipzig, 1–765.

Mimura, T and Shimmen, T (1994). Characterization of the Ca^{2+}-dependent Cl^{-} efflux in perfused *Chara* cells. *Plant Cell Physiology* 5, 793–800.

Müller, G, Irion, G, and Förstner, U (1972). Formation and diagenesis of inorganic Ca-Mg carbonates in the lacustrine environment. *Die Naturwissenschaften* 59, 158–164.

Murphy, T P, Hall, K J, and Yesaki, I (1983). Coprecipitation of phosphate with calcite in a naturally eutrophic lake. *Limnology and Oceanography* 28, 58–69.

Nielsen, R (2003). *Chara aspera* Willd. 1809. In: Charophytes of the Baltic Sea. Ed. by H Schubert and I Blindow. Gantner, Ruggell, 42–52.

Nõges, P, Tuvikene, L, Feldmann, T, Tõnno, I, Künnap, H, Luup, H, Salujõe, J, and Nõges, T (2003). The role of charophytes in increasing water transparency: a case study of two shallow lakes in Estonia. *Hydrobiologia* 506-509, 567–573.

Oksanen, J, Guillaume Blanchet, F, Friendly, M, Kindt, R, Legendre, P, McGlinn, D, Minchin, P R, O'Hara, R B, Simpson, G L, Solymos, P, Stevens, M H H, Szoecs, E, and Wagner, H (2017). vegan: Community Ecology Package. R package version 2.4-3.

Olguín, E J (2003). Phycoremediation: key issues for cost-effective nutrient removal processes. *Biotechnology Advances* 22, 81–91.

Oswald, W J (1988). Large scale culture systems: engineering aspects. In: Microalgal biotechnology. Ed. by M Borowitzka and L Borowitzka. Cambridge University, Cambridge, 357–392.

Otsuki, A and Wetzel, R G (1972). Coprecipitation of phosphate with carbonates in a marl lake. *Limnology and Oceanography*, 763–767.

Ozimek, T and Kowalczewski, A (1984). Long-term changes of the submersed macrophytes in eutrophic Lake Mikołajskie (North Poland). *Aquatic Botany* 19, 1–11.

Patzelt, L (2017). Untersuchungen zum Akkumulationsverhalten ausgewählter Schwermetalle an Armleuchteralgen, Bachelor thesis. *University of Jena*, 1–65.

Pedersen, O, Colmer, T D, and Sand-Jensen, K (2013). Underwater photosynthesis of submerged plants – recent advances and methods. *Frontiers in Plant Science* 4, 1–19.

Pełechaty, M, Pukacz, A, Apolinarska, K, Pełechata, A, and Siepak, M (2013). The significance of *Chara* vegetation in the precipitation of lacustrine calcium carbonate. *Sedimentology* 60, 1017–1035.

Pentecost, A (1984). The growth of *Chara globularis* and its relationship to calcium carbonate deposition in Malham Tarn. *Field Studies* 6, 53–58.

Pentecost, A, Andrews, J E, Dennis, P F, Marca-Bell, A, and Dennis, S (2006). Charophyte growth in small temperate water bodies: Extreme isotopic disequilibrium and implications for the palaeoecology of shallow marl lakes. *Palaeogeography, Palaeoclimatology, Palaeoecology* 240, 389–404.

Pieczyńska, E, Ozimek, T, and Rybak, J I (1988). Long-term changes in littoral habitats and communities in Lake Mikołajskie (Poland). *Internationale Revue der gesamten Hydrobiologie und Hydrographie* 73, 361–378.

Porra, R J, Thompson, W A, and Kriedemann, P E (1989). Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochimica et Biophysica Acta* 975, 384–394.

Pukacz, A, Pełechaty, M, and Frankowski, M (2014a). Carbon dynamics in a hardwater lake: effect of charophyte biomass on carbonate deposition. *Polish Journal of Ecology* 62, 695–705.

Pukacz, A, Pełechaty, M, and Frankowski, M (2016a). Depth-dependence and monthly variability of charophyte biomass production: consequences for the precipitation of calcium carbonate in a shallow *Chara*-lake. *Environmental Science and Pollution Research* 23, 22433–22442.

Pukacz, A, Pełechaty, M, Frankowski, M, Kowalski, A, and Zwijacz-Koszałka, K (2014b). Seasonality of water chemistry, carbonate production, and biometric features of two species of *Chara* in a shallow clear water lake. *Scientific World Journal*. DOI: 10.1155/2014/167631.

Pukacz, A, Pełechaty, M, Frankowski, M, and Pronin, E (2016b). Dry weight and calcium carbonate encrustation of two morphologically different *Chara* species: a comparative study from different lakes. *Oceanological and Hydrobiological Studies* 45, 377–387.

R Core Team (2017). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria.

Raven, J A, Smith, F A, and Walker, N A (1986). Biomineralization in the Charophyceae sensu lato. In: Biomineralization in Lower Plants and Animals. The Systematics Association. Ed. by B S C Leadbeater and R Riding. Clarendon Press, Oxford, 125–139.

Ray, S, Klenell, M, Choo, K S, Pedersen, M, and Snoeijs, P (2003). Carbon acquisition mechanisms in *Chara tomentosa*. *Aquatic Botany* 76, 141–154.

Remmert, H (1978). Ökologie-Ein Lehrbuch. Spriner, Berlin, p. 203.

Rushdi, A I, Pytkowicz, R M, Suess, E, and Chen, C T (1992). The effects of magnesium-to-calcium ratios in artificial seawater, at different ionic products, upon the induction time, and the mineralogy of calcium carbonate: a laboratory study. *Geologische Rundschau* 81, 571–578.

Rybak, Michał, Kołodziejczyk, A, Joniak, T, Ratajczak, I, and Gabka, M (2017). Bioaccumulation and toxicity studies of macroalgae (Charophyceae) treated with aluminium: Experimental studies in the context of lake restoration. *Ecotoxicology and Environmental Safety* 145, 359–366.

Sand-Jensen, K, Jensen, R S, Gomes, M, Kristensen, E, Martinsen, K T, Kragh, T, Baastrup-Spohr, L, and Borum, J (2018). Photosynthesis and calcification of charophytes. *Aquatic Botany* 149, 46–51.

Santi, S, Locci, G, Pinton, R, Cesco, S, and Varanini, Z (1995). Plasma membrane H^+-ATPase in maize roots induced for NO_3^- uptake. *Plant Physiology* 109, 1277–1283.

Scheffer, M (2001). Alternative attractors of shallow lakes. *The Scientific World* 1, 254–263.

Scheffer, M, Hosper, S, Meijer, M, Moss, B, and Jeppesen, E (1993). Alternative equilibria in shalow lakes. *Trends in Ecology and Evolution* 8, 275–279.

Schneider, S C and Nizzetto, L (2012). Bioconcentration and intracellular storage of hexachlorobenzene in charophytes and their potential role in monitoring and remediation actions. *Environmental Science and Technology* 46, 12427–12434.

Schöbe, S (2017). Praktikumsbericht: Methodenadaption zur chemischen Abtrennung von Kalziumkarbonat kalzifizierter Characeen als Grundlage für eine sequenzielle Extraktion, Helmholtz-Zentrum für Umweltforschung, Magdeburg, 1–10.

Schöler, A, Zaharieva, I, Zimmermann, S, Wiechen, M, Manke, A-M, Kurz, P, Plieth, C, and Dau, H (2014). Biogenic manganese-calcium oxides on the cell walls of the algae *Chara corallina*: elemental composition, atomic structure, and water-oxidation catalysis. *European Journal of Inorganic Chemistry*, 780–790.

Schreck, P, Wennrich, R, Stärk, H J, Schubert, M, and Weiß, H (2004). Mansfeld – the contribution of a mining-affected catchment area to regional riverine pollution. *Helmholtz-Zentrum für Umweltforschung – UFZ-Bericht 18/2004, Leipzig*, pp. 169–170.

Schubert, H and Blindow, I (2003). Charophytes of the Baltic Sea. Gantner, Ruggell, 1–326.

Schubert, H, Blindow, I, Bueno, N C, Casanova, M T, Pełechaty, M, and Pukacz, A (2018a). Ecology of charophytes – permanent pioneers and ecosystem engineers. *Perspectives in Phycology* 5, 61–74.

Schubert, H, Blindow, I, Schories, D, Mages, M, von Tümpling, W, and Woelfl, S (2018b). Biogeography of Chilean Charophytes – determined by climate or by water chemistry? *Botany Letters* 165, 129–145.

Schubert, H, Schories, D, Schneider, B, and Selig, U (2017). Brackish water as an environment. In: Biological Oceanography of the Baltic Sea. Ed. by P Snoeijs-Leijonmalm, H Schubert, and T Radziejewska. Springer, Dordrecht, 3–22.

Schütte, M (2003). Wuchs- und Lebensformen bei Armleuchteralgen (*Chara aspera*) in Abhängigkeit von Salinität und Tiefe. *Diploma thesis, University of Greifswald, Greifswald*.

Schützendübel, A and Polle, A (2002). Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botan* 53, 1351–1365.

Shimmen, T and MacRobbie, E A C (1987). Characterization of two proton transport systems in the tonoplast of plasmalemma-permeabilized *Nitella* cells. *Plant and Cell Physiology* 28, 1023–1031.

Siong, K and Asaeda, T (2009). Calcite encrustation in macro-algae *Chara* and its implication to the formation of carbonate-bound cadmium. *Journal of hazardous materials* 167, 1237–1241.

Smith, F A (1967). Rates of photosynthesis in characean cells II. Photosynthetic $^{14}CO_2$ fixation and ^{14}C- bicarbonate uptake by characean cells. *Journal of Experimental Botany* 19, 207–217.

Smith, F A (1968). Rates of photosynthesis in characeaen cells: II. Photosynthetic $^{14}CO_2$ fixation and ^{14}C-bicarbonate uptake by characean cells. *Journal of Experimental Botany* 19, 207–217.

Smith, F A and Walker, N A (1980). Photosynthesis by aquatic plants: effects of unstirred layers in relation to assimilation of CO_2 and HCO_3^- and to carbon isotopic discrimination. *New Phytologist* 86, 245–259.

Sooksawat, N, Meetam, M, Kruatrachue, M, Pokethitiyook, P, and Inthorn, D. (2016). Equilibrium and kinetic studies on biosorption potential of charophyte biomass to remove heavy metals from synthetic metal solution and municipal wastewater. *Bioremediation Journal* 20, 240–251.

Sooksawat, N, Meetam, M, Kruatrachue, M, Pokethitiyook, P, and Nathalang, K (2013). Phytoremediation potential of charophytes: Bioaccumulation and toxicity studies of cadmium, lead and zinc. *Journal of Environmental Sciences* 25, 596–604.

Sorrell, B K, Hawes, I, Schwarz, A M, and Sutherland, D (2001). Inter-specific differences in photosynthetic carbon uptake, photosynthate partitioning and extracellular organic carbon release by deep-water characean algae. *Freshwater Biology* 46, 453–464.

Spear, D G, Barr, J K, and Barr, C E (1969). Localization of hydrogen ion and chloride ion fluxes in *Nitella*. *The Journal of General Physiology* 54, 397–413.

Stewart, K D and Mattox, K R (1975). Comparative cytology, evolution and classification of the green algae with some consideration on the origin of other organisms with chlorophylls *a* and *b*. *Botanical Review* 41, 104–135.

Taylor, G J, McDonald-Stephens, J L, Hunter, D B, Bertsch, P M, Elmore, D, Rengel, Z, and Reid, R J (2000). Direct measurement of aluminum uptake and distribution in single cells of *Chara corallina*. *Plant Physiology* 123, 987–996.

Torn, K, Martin, G, and Munsterhjelm, R (2003). *Chara tomentosa* L. 1753. In: Charophytes of the Baltic Sea. Ed. by H Schubert and I Blindow. Gantner, Ruggell, 131–141.

Triboit, F, Laffont-Schwob, I, Demory, F, Soulié-Märsche, I, Rabier, J, and Despréaux Mand Thiéry, A (2010). Heavy metal lability in porewater of highway detention pond sediments in south-eastern france in relation to submerged vegetation. *Water, Air, and Soil Pollution* 209, 229–240.

Ullrich, C I, Witt, F G, Aparicio, P J, Ullrich, W R, and Lazarova, J (1998). Nitrate uptake and extracellular alkalinization by the green alga *Hydrodictyon reticulatum* in blue and red light. *Journal of Experimental Botany* 49, 1157–1162.

Urbaniak, J (2010). Estimation of carbonate and element content in charophytes – methods of determination. *Polish Journal of Environmental Studies* 19, 413–417.

Van den Berg, M S, Coops, H, Meijer, M L, Scheffer, M, and Simons, J (1998a). Clearwater associated with a dense *Chara* vegetation in the shallow and turbid Lake Veluwemeer, the Netherlands. In: The Structuring Role of Submerged Macrophytes in Lakes. Ed. by E Jeppesen, M Søndergaard, M Søndergaard, and K Christoffersen. Springer, New York, 339–352.

Van den Berg, M S, Coops, H, Simons, J, and Pilon, J (2002). A comparative study of the use of inorganic carbon resources by *Chara aspera* and *Potamogeton pectinatus*. *Aquatic Botany* 72, 219–233.

Van den Berg, M S, Scheffer, M, Coops, H, and Simons, J (1998b). The role of characean algae in the management of eutrophic shallow lakes. *Journal of Phycology* 34, 750–756.

Wahlstedt, L J (1862). Bidrag till kännedomen om de skandinaviska arterna af växtfamiljen Characeae. Academiska Afhandlingar, Lund, Sweden.

Wahlstedt, L J (1875). Monografi öfver Sveriges och Norges characeer. *Boktryckeri-Aktie-Bolaget, Christianstad, Sweden*, 37 pp.

Wang, H, Ni, L, and Xie, P (2013). The mitigating effect of calcification-dependent of utilization of inorganic carbon of *Chara vulgaris* Linn on NH_4-N toxicity. *Chemosphere*, 373–379.

Wetzel, R G (1975). Limnology. Lake and River Ecosystems. Elsevier, London.

Winter, U and Kirst, G O (1990). Salinity response of a freshwater charophyte, *Chara vulgaris*. *Plant, Cell and Environment* 13, 123–134.

Winter, U and Kirst, G O (1991a). Partial turgor pressure regulation in *Chara canescens* and its implications for a generalized hypothesis of salinity response in Charophytes. *Botanica Acta* 104, 37–46.

Winter, U and Kirst, G O (1991b). Vacuolar sap composition during sexual reproduction and salinity stress in Charophytes. *Bulletin de la Société Botanique de France. Actualités Botaniques* 138, 85–93.

Winter, U and Kirst, G O (1992). Turgor pressure regulation in *Chara aspera* (Charophyta): the role of sucrose accumulation in fertile and sterile plants. *Phycologia* 31, 240–245.

Winter, U, Kirst, G O, Grabowski, V, Heinemann, U, Plettner, I, and Wiese, S (1999). Salinity tolerance in *Nitellopsis obtusa*. *Australian Journal of Botany* 47, 337–346.

Winter, U, Meyer, M I B, and Kirst, G O (1987). Seasonal changes of ionic concentrations in the vacuolar sap of *Chara vulgaris* L. growing in a brackish water lake. *Oecologia* 74, 122–127.

Winter, U, Soulié-Märsche, I, and Kirst, G O (1996). Effects of salinity on turgor pressure and fertility in *Tolypella* (Characeae). *Plant Cell and Environment* 19, 869–879.

Wood, R D (1962). New combinations and taxa in the revision of *Characeae*. *Taxon* 11, 7–25.

Wood, R D and Imahori, K (1965). A revision of the *Characeae*: Monograph of the *Characeae*. Cramer, Weinheim, p. 159.

List of Abbreviations

AFDW	ash free dry weight
ANOVA	analysis of variance
BW	brackish water
chl	chlorophyll
car	carotenoid
cond	conductivity
DIC	dissolved inorganic carbon
DW	dry weight
ETR	electron transport rate
f	fertile
FW	freshwater
HSD	honestly significant difference
I	internode
ICP-OES	inductively coupled plasma-optic emission spectroscopy
LOI	loss of ignition
LSD	least significant difference
n	number
nf	non-fertile
NMDS	non-metric multidimensional scaling
PAM	pulse amplitude modulation
PCA	principal component analysis
ROS	reactive oxygen species
SA	spring – autumn
SD	standard deviation
SW	summer – winter
TIC	total inorganic carbon
TP	total phosphorus
W	whorl

Species

asp	*Chara aspera*
can	*C. canescens*
fle/opa	*Nitella flexilis/opaca*
glo	*C. globularis*
his	*C. hispida*
obt	*Nitellopsis obtusa*
sub	*C. subspinosa*
tom	*C. tomentosa*

Sampling sites

Krüselinsee	Krü
Lützlower See	Lüt
Angersdorfer Teiche	Ang
Asche	Asc
Bruchwiesen	Bru
Sabinensee	Sab
Gottssee	Got
Kölpinsee	Köl
Parsteiner See	Par
Krüselinsee	Krü
Krankesjön	Kra
Börringesjön	Bör
Lyngsjön	Lyn
Räpplinge	Räp
Greby	Gre
Gällerskullaviken	Gäl
Hålviksfjärden	Hål
Loftahammar	Lof
Klumpudden	Klu
Edenryd	Ede
Sibbaboda	Sib
Schlüsselstollen	S
$\text{Saale}_{\text{above}}$	Sa
$\text{Saale}_{\text{below}}$	Sb
Altarm	A

List of Figures

List of Tables

Appendix

Table A1: Comparison of plant element content (mean ± SD) of species sampled in June from Krüselinsee and Lützlower See. Ca, K, Na, Mg, and P contents are presented as g kg^{-1} DW. Different letters indicate significant differences (Tukey HSD post-hoc test, $p < 0.05$) in element content of DW, n = number of sample size.

site	n	Ca	Fe	K	Mg	P
Krüselinsee	6	259.3 ± 27.0 a	0.04 ± 0.05 a	10.5 ± 2.4 a	4.0 ± 0.5 a	0.8 ± 0.4 a
Lützlower See	6	258.3 ± 33.5 a	0.09 ± 0.04 b	8.9 ± 2.3 a	4.7 ± 0.5 b	0.7 ± 0.4 a

Table A2: Comparison of plant element content (mean ± SD) of species sampled in July from Krüselinsee and Lützlower See. Ca, K, Na, Mg, and P contents are presented as g kg^{-1} DW. Different letters indicate significant differences (Tukey HSD post-hoc test, $p < 0.05$) in element content of DW, n = number of sample size.

site	n	Ca	Fe	K	Mg	P
Krüselinsee	6	254.8 ± 21.7 a	0.02 ± 0.02 a	8.7 ± 1.4 a	3.6 ± 0.5 a	0.8 ± 0.3 a
Lützlower See	6	267.8 ± 20.8 a	0.07 ± 0.03 b	8.7 ± 2.9 a	4.5 ± 0.4 b	0.6 ± 0.3 a

Table A3: Comparison of carbonate content (mean ± SD) based on DW (%) of *C. canescens* (can), *C. hispida* (his), *C. subspinosa* (sub), and *C. tomentosa* (tom) from different sampling sites (Angersdorfer Teiche, Asche, Bruchwiesen, Krüselinsee, and Lützlower See). Species were analysed in parts of thallus; first to third (1-3), forth to sixth (4-6), seventh to ninth (7-9) whorls and internodes. Sample size was n = 7 for the LOI analysis. Different letters indicate significant difference within the same sampling site (Fisher LSD post-hoc test, $p < 0.05$).

site	species	part	carbonate
Angersdorfer Teiche	can	1-3	14.3 ± 1.2 a
		4-6	22.4 ± 1.4 b
		7-9	30.7 ± 2.0 c
	his	1-3	35.7 ± 0.6 d
		4-6	40.0 ± 1.0 e
		7-9	42.4 ± 0.9 f
Asche	tom	1-3	20.5 ± 0.9 a
		4-6	24.6 ± 0.9 b
		7-9	24.1 ± 1.6 b
Bruchwiesen	his	1-3	27.2 ± 1.2 a
		4-6	30.7 ± 1.7 b
		7-9	32.4 ± 0.6 b
Krüselinsee	sub	1-3	38.6 ± 0.7 a
		4-6	43.8 ± 0.8 b
		7-9	43.6 ± 1.2 b
	tom	1-3	32.4 ± 1.5 c
		4-6	39.9 ± 1.2 a
		7-9	38.4 ± 1.1 a
Lützlower See	sub	1-3	41.2 ± 0.4 ab
		4-6	42.3 ± 0.6 a
		7-9	40.7 ± 0.8 abc
	tom	1-3	38.7 ± 0.9 c
		4-6	39.3 ± 2.1 bc
		7-9	40.4 ± 0.6 abc

Table A4: Water chemistry data of sampling sites in fresh- and brackish waters. Ion concentrations are given in mg L^{-1}, conductivity in µS cm^{-1}. Salinity is derived from conductivity data and serves as a rough estimation without correction for ion anomalies; K^+/Na^+ mass ratio is calculated from weight.

habitat	site	Ca^{2+}	Cl^-	K^+	Mg^{2+}	Na^+	$SO_4{}^{2-}$	TIC	TP	pH	cond	salinity	K^+/Na^+
FW	Sabinensee	45.5	30	6.53	10.1	14.6	30.5	29.6	0.027	8.3	0.38	0	0.45
	Gottssee	58	13.5	0.53	7.3	8.7	51.7	26.7	0.025	7.8	0.37	0	0.06
	Kölpinsee	56.5	18.3	3.33	7.7	10.1	59.2	25	0.026	8.4	0.39	0	0.33
	Parsteiner See	47.8	42.5	11	16.1	17.5	66.9	25.6	0.023	8.1	0.47	0	0.63
	Krüselinsee	43.2	18.3	3.34	7.9	10.7	29.3	27.5	0.025	8.3	0.34	0	0.31
	Krankesjön	31.4	18.9	0.92	4.6	13.1	25.8	15.5	0.032	8.8	0.25	0	0.07
	Börringesjön	56.7	34.8	2.98	5.56	19.6	17.7	34.3	0.123	8.7	0.38	0	0.15
	Lyngsjön	58.4	17.7	1.24	1.8	7.9	22.9	38.5	0.055	8.1	0.47	0	0.16
	Räpplinge	40.6	34.5	1.74	1.4	23.2	2.6	25	0.021	8.5	0.31	0	0.08
	Greby	49.4	99.7	1.15	1.4	58.8	10.2	31.3	0.019	8.2	0.54	0	0.02
BW	Gällerskullaviken	79	2400	57	181	1550	365	15.7	0.016	7.7	8.2	4.5	0.04
	Hålviksfjärden	96,3	3140	75.2	232	1980	462	19	0.031	7.9	10.4	5.9	0.04
	Loftahammar	105	3610	86.9	270	2260	525	20	0.039	8.3	11.7	6.7	0.04
	Klumpudden	109	3730	90.8	272	2300	556	20.5	0.019	8.4	12.2	7	0.04
	Edenryd	121	4210	105	309	2560	617	21.9	0.032	8.7	13.4	7.7	0.04
	Sibbaboda	121	4240	103	304	2580	595	23.2	0.019	8.9	13.5	7.8	0.04

Table A5: Comparison of water chemistry data of Asche, Lützlower See, Saaleabove, Saalebelow, Altarm, Altarm:Schlüsselstollen, and Schlüsselstollen. Listed are Ca^{2+}, Cd^{2+}, Cu^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Pb^{2+}, Zn^{2+}, TP concentrations (mg L^{-1}), pH, and conductivity (mS cm^{-1}).

site	Ca^{2+}	Cd^{2+}	Cu^{2+}	K^+	Mg^{2+}	Mn^{2+}	Na^+	Pb^{2+}	Zn^{2+}	TP	pH	cond	salinity
Asche	321.2	< 0.002	< 0.005	38.2	473.6	< 0.007	598.6	< 0.05	< 0.009	< 0.06	7.6	5.0	3.0
Lützlower See	114.5	< 0.002	< 0.005	4.9	25.2	< 0.007	35.3	< 0.05	< 0.009	< 0.06	8.5	0.75	0.2
Saaleabove	133.0	< 0.002	0.005	15.6	33.8	< 0.007	155.6	< 0.05	< 0.009	0.114	8.1	1.4	0.6
Saalebelow	141.0	< 0.002	< 0.005	16.6	36.1	< 0.007	205.2	< 0.05	0.058	0.145	8.0	1.6	0.8
Altarm	77.9	< 0.002	0.008	15.3	36.2	< 0.007	178.0	< 0.05	< 0.009	0.687	9.5	1.3	0.6
Altarm:Schlüsselstollen(3:1)	232.2	0.008	0.041	41.9	88.5	0.058	1963.2	0.033	2.9	0.412	8.9	9.1	5.9
Schlüsselstollen	696.4	0.027	0.148	136.2	229.7	0.218	8460	0.194	10.9	< 0.06	7.4	26.4	21.4

Table A6: Comparison of physiological parameters of electron transport rates (ETR_{max}) in µmol electrons $m^{-2}s^{-1}$, $Yield_{dark}$, chlorophyll *a* (chl a), *b* (chl b), and carotenoid (car) contents in fresh weight (µg g^{-1}), chl a/b-ratio, chl a/car-ratio, and carbonate content (%) of DW. Species are abbreviated as first three letters: *C. subspinosa* (sub), and *C. tomentosa* (tom). Approaches are combined with sediment and water from Asche (H1H1), Lützlower See (H2H2), $Saale_{above}$ (SaSa), $Saale_{below}$ (SbSb), Altarm (AA), Schlüsselstollen (SS). Sediment from Asche (H1) and Lützlower See (H2) were also combined with water of Altarm:Schlüsselstollen (AS) and Schlüsselstollen (S). Different letters indicate significant difference (Tukey HSD post-hoc test, $p < 0.05$) for the given parameters, n = number of replicates.

level	n	ETR_{max}	$Yield_{dark}$	chl a	chl b	car	chl a/b	chl a/car	carbonate
species									
sub	40	20.0 ± 13.1 b	0.556 ± 0.291 a	181.6 ± 82.9 a	63.4 ± 25.3 a	41.6 ± 19.8 a	2.76 ± 0.47 a	4.63 ± 1.10 a	35.3 ± 6.3 a
tom	80	31.5 ± 18.7 a	0.599 ± 0.260 a	169.4 ± 78.4 a	58.0 ± 23.7 a	230.9 ± 151.4 b	2.85 ± 0.44 a	0.98 ± 0.58 b	29.45± 8.6 b
approach									
H1tom	10	38.9 ± 7.5 ab	0.730 ± 0.019 ab	238.8 ± 50.2 ab	78.5 ± 17.4 a	454.1 ± 197.6 a	3.06 ± 0.20 ab	0.53 ± 0.09 e	21.9 ± 3.1 e
H2sub	10	31.1 ± 9.6 bcd	0.717 ± 0.051 ab	183.7 ± 28.4 bcd	65.2 ± 10.9 abc	41.3 ± 5.7 d	2.83 ± 0.12 bcd	4.45 ± 0.49 a	34.0 ± 4.8 a
H2tom	10	55.3 ± 12.6 a	0.744 ± 0.028 ab	113.5 ± 24.4 efg	40.2 ± 9.2 d	203.3 ± 46.2 ab	2.84 ± 0.10 bcd	0.58 ± 0.14 de	37.6 ± 4.7 de
AA	9	38.8 ± 12.2 abc	0.709 ± 0.040 ab	254.0 ± 42.1 a	81.4 ± 13.1 a	191.4 ± 144.5 bc	3.13 ± 0.15 a	2.46 ± 2.11 abc	26.6 ± 6.8 abc
H1H1	9	23.4 ± 12.2 cde	0.673 ± 0.206 ab	221.9 ± 44.4 abc	77.8 ± 16.5 a	218.4 ± 138.5 ab	2.86 ± 0.13 abcd	1.55 ± 1.11 bcde	33.8 ± 7.3 bcde
H1S	9	9.5 ± 9.2 efg	0.264 ± 0.184 cd	66.5 ± 42.7 g	27.9 ± 18.8 d	36.8 ± 27.3 d	2.42 ± 0.41 cde	2.40 ± 1.45 abc	40.0 ± 6.7 abc
H1AS	9	30.8 ± 15.7 bcd	0.645 ± 0.160 ab	162.1 ± 58.5 cde	48.3 ± 13.5 cd	153.6 ± 123.8 bc	3.30 ± 0.63 ab	1.82 ± 1.52 abcd	29.1 ± 7.4 abcd
H2H2	9	23.5 ± 6.0 def	0.753 ± 0.017 a	240.1 ± 49.1 ab	81.7 ± 16.3 a	228.8 ±188.0 ab	2.94 ± 0.16 ab	2.34 ± 2.30 abc	25.9 ± 9.9 abc
H2S	9	0.75 ± 1.2 g	0.052 ± 0.055 d	63.7 ± 32.2 g	33.3 ± 13.4 d	24.4 ± 18.4 d	1.87 ± 0.30 e	3.94 ± 2.49 ab	33.8 ± 7.5 ab
H2AS	9	34.2 ± 11.9 bcd	0.710 ± 0.030 bc	158.3 ± 37.6 cde	54.4 ± 14.0 bcd	177.4 ± 116.8 bc	2.92 ± 0.14 ab	1.58 ± 1.41 cde	27.5 ± 9.1 abcd
SaSa	9	33.8 ± 9.0 bcd	0.750 ± 0.016 a	249.2 ± 53.7 ab	82.1 ± 17.5 a	208.3 ± 154.9 ab	3.04 ± 0.11 ab	2.19 ± 1.84 abcd	30.5 ± 6.7 abcd
SbSb	9	33.5 ± 6.5 bcd	0.749 ± 0.013 ab	219.8 ± 49.0 abcd	70.7 ± 15.5 ab	153.8 ± 103.1 bc	3.11 ± 0.07 a	2.31 ± 1.91 abc	37.6 ± 7.0 abc
SS	9	1.1 ± 1.1 fg	0.055 ± 0.042 d	82.0 ± 33.5 fg	35.4 ± 13.0 d	68.4 ± 67.0 cd	2.32 ± 0.35 de	2.50 ± 2.22 abc	29.9 ± 6.6 abc
water									
A	9	38.8 ± 12.2 a	0.709 ± 0.040 bc	254.0 ± 42.1 a	81.4 ± 13.1 a	191.4 ± 144.5 ab	3.13 ± 0.15 a	2.46 ± 2.11 ab	26.6 ± 6.8 b
AS	18	32.5 ± 13.6 a	0.678 ± 0.116 c	160.2 ± 47.7 b	51.4 ± 13.7 b	165.5 ± 117.4 b	3.11 ± 0.48 ab	1.70 ± 1.43 ab	27.5 ± 8.1 b
H1	9	31.5 ± 12.6 a	0.703 ± 0.141 abc	230.8 ± 47.0 a	78.1 ± 16.5 a	342.4 ± 170.1 a	2.97 ± 0.19 ab	1.02 ± 0.907 b	32.7 ± 8.2 ab
H2	9	37.1 ± 16.8 a	0.738 ± 0.037 ab	177.0 ± 62.3 b	61.7 ± 21.0 b	155.4 ± 134.1 b	2.87 ± 0.13 b	2.46 ± 2.07 ab	37.1 ± 7.3 a
S	27	3.8 ± 6.6 b	0.124 ± 0.149 d	70.7 ± 36.0 c	32.2 ± 15.1 c	43.2 ± 45.5 c	2.21 ± 0.42 c	2.95 ± 2.14 a	28.3 ± 8.1 b
Sa	9	33.8 ± 9.0 a	0.750 ± 0.016 a	249.2 ± 53.7 a	82.1 ± 17.5 a	208.3 ±154.9 ab	3.036 ± 0.11 ab	2.19 ± 1.84 ab	30.5 ± 6.7 ab
Sb	9	33.5 ± 6.5 a	0.749 ± 0.014 ab	219.8 ± 49.1 ab	70.7 ± 15.5 ab	153.8 ± 103.1 b	3.11 ± 0.07 a	2.31 ± 1.91 ab	29.9 ± 6.61 ab

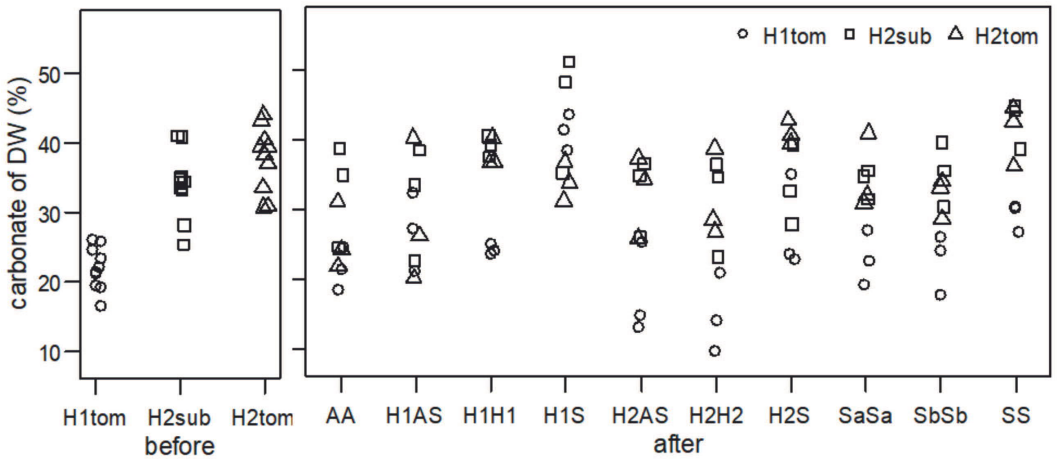

Figure A1: Carbonate content based on DW (%) of plants (LOI analysis) before and after incubation. *C. subspinosa* (sub) and *C. tomentosa* (tom) from Asche (H1) and Lützlower See (H2) were planted in different sediment/water combination: sediment and water from Asche (H1H1), Lützlower See (H2H2), $Saale_{above}$ (SaSa), $Saale_{below}$ (SbSb), Altarm (AA), Schlüsselstollen (SS). Sediment from Asche (H1) and from Lützlower See (H2) were also combined with Altarm:Schlüsselstollen (AS) and Schlüsselstollen (S) water. Species are presented as symbols, see legend.

Table A7: Comparison of ash free dry weight (AFDW) and carbonate contents of dried sediment (%) before and after incubation. Sediments are abbreviated as Altarm (A), Asche (H1), Lützlower See (H2), Schlüsselstollen (S), $Saale_{above}$ (Sa), and $Saale_{below}$ (Sb) before incubation. After incubation with Asche (H1H1), Lützlower See (H2H2), $Saale_{above}$ (SaSa), $Saale_{below}$ (SbSb), Altarm (AA), and Schlüsselstollen (SS) waters, approaches are abbreviated. Sediment from Asche (H1) and from Lützlower See (H2) were combined with Altarm:Schlüsselstollen (AS) and Schlüsselstollen (S) water. Different letters indicate significant difference (Fisher LSD post-hoc test, $p < 0.05$), n = number of approach size.

	approach	n	AFDW	carbonate
before	A	10	19.2 ± 1.6 d	5.5 ± 0.5 e
	H1	10	27.7 ± 1.8 bc	32.3 ± 1.3 bc
	H2	10	0.3 ± 0.0 i	1.9 ± 0.1 i
	S	10	3.8 ± 0.3 g	7.2 ± 0.6 d
	Sa	10	10.9 ± 0.4 f	3.8 ± 0.8 f
	Sb	10	8.7 ± 0.2 e	3.5 ± 0.5 f
after	AA	9	18.5 ± 0.8 d	5.8 ± 0.6 e
	H1H1	9	29.5 ± 1.5 a	29.4 ± 1.6 c
	H1S	9	24.5 ± 2.6 c	38.9 ± 1.6 a
	H1AS	9	28.6 ± 0.6 ab	33.7 ± 0.9 ab
	H2H2	9	0.4 ± 0.1 i	2.1 ± 0.1 h
	H2S	9	0.8 ± 0.1 h	2.5 ± 0.2 g
	H2AS	9	0.5 ± 0.1 h	2.0 ± 0.2 hi
	SaSa	9	11.8 ± 1.0 f	3.5 ± 0.5 f
	SbSb	9	7.6 ± 0.9 e	2.7 ± 0.3 g
	SS	9	4.2 ± 0.4 g	6.8 ± 0.7 d

Acknowledgement

First, the support by “Promotionsstipendiumprogramm of Deutsche Bundesstiftung Umwelt” (DBU) is gratefully acknowledged. It is a pleasure for me to be a fellow.

Further gratitude is owed to the “Professorinnenprogramm II of the University of Rostock” for its support.

Foremost, I would like to thank Prof. Dr. Hendrik Schubert for the supervision of my dissertation, for the helpful discussions and the resulting ideas. I am very grateful for your support.

PD Dr. Wolf von Tümpling, thank you for the cooperation with the Helmholtz Centre for Environmental Research (UFZ) in Magdeburg and for the support with the chemical part of this study including the analyses of the water samples.

PD Dr. Irmgard Blindow, I would like to thank you for the good cooperation and the pleasant time in València and Hiddensee.

Dr. Christian Porsche, thank you for your help with the photosynthesis measurements, the statistical analyses and for introducing me to QGIS.

Dr. Petra Nowak, I would like to thank you for the adventure of finding the sampling site Asche, and of course for driving me.

Dr. Margarete Kalin, thank you for the supporting ideas and interesting discussions on bioremediation.

Britta Balz, Elena Heilmann (AUF, University of Rostock) and Dorothee Ohlwein (UFZ, Magdeburg), thank you for providing technical support for the analyses of biomass and water.

Levke Henningsen, Leila Patzelt and Stefanie Schöbe, thank you for performing your theses and work experiences with encrusted charophytes. You have done a great job!

I would further like to thank Baltic TRANSCOAST, for being an associated PhD student in the project.

Thanks also to those who helped proof-reading: Christiane Volkmann, Steffi Spielberg and Vicky Kelly.

The Aquatic Ecology, thanks to all of you for the pleasant time in the working group.

Thank you!

Declaration of Authorship

I hereby certify that the thesis I am submitting was written only with the assistance and literature cited in the text. Only the sources cited have been used in this work. Parts that are direct quotes or paraphrases are identified as such. The figures and photographs in this work have been prepared by me, if not labelled otherwise.
The thesis has not been previously submitted whether to the University of Rostock or to any other university.

Rostock, 29.06.2018

Anne Herbst

Personal information

Adress	Oldendorpstraße 16, 18059 Rostock
Contact	anne.herbst2@uni-rostock.de
Date/place of birth	24.10.1988 in Braunschweig

Education

07/2015 – 10/2018 **PhD Student**, *University of Rostock, Faculty of Mathematics and Natural Sciences, Aquatic Ecology*, Topic: Encrustation of charophytes, Scholarship from the German Federal Environmental Foundation (DBU).

2012 – 2014 **Master Marine Biology**, *University of Rostock, Faculty of Mathematics and Natural Sciences, Aquatic Ecology*, Thesis: Nutrient uptake in the red alga *Delesseria sanguinea* (Hudson) Lamouroux from the Mecklenburg Bight (Baltic Sea), grade: 1.5.

2009 – 2012 **Bachelor Biology**, *Friedrich Schiller University Jena, Faculty of Biology and Pharmacy, General Botany*, Thesis: Localisation studies of animal-like cryptochrome in *Chlamydomonas reinhardtii*, grade: 1.6.

Work experiences

10/2014 – 03/2015 **Research assistant**, *University of Rostock, Faculty of Mathematics and Natural Sciences, Aquatic Ecology.*

10/2013 – 03/2014 **Scientific assistant**, *Leibniz Institute for Baltic Sea Research Warnemünde, Marine Geology.*

10/2010 – 03/2012 **Student assistant**, *Max Planck Institute for Chemical Ecology Jena, Entomology and Biochemistry.*

Practical training

10/2017 **Hyytiälä/Tvärminne in Finland**, summer school: subsurface and coastal water dynamics, peat ecology.

08/2017 **German Federal Environmental Foundation**, Helgoland, seminar: microplastics in aquatic systems.

08/2015 **Sven Lovén Centre for Marine Sciences in Sweden**, *University of Gothenburg*, int. course in scientific diving and documentation techniques.

07/2013 **European Scientific Diver**, certified by Kommission Forschungstauchen Deutschland.

10/2011 **HYDRA Institute for Marine Science in Italy**, Island of Elba, field course in marine biology diving skills.

09/2011 **Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures**, training in the department of Human and Animal Cell Lines.

Publications

Herbst, A., Henningsen, L., Schubert, H., Blindow, I., 2018. Encrustations and element composition of charophytes from fresh or brackish water sites — habitat- or species-specific differences?, Aquatic Botany 148: 29-34.

Herbst, A., von Tümpling, W., Schubert, H., 2018. The seasonal effects on the encrustation of charophytes in two hard-water lakes, Journal of Phycology, 54: 630-637.

Conferences and presentations

Herbst, A., Schubert, H., 2017. Calcification of charophytes – pattern and ion composition dependency, 21th Meeting of the Group of European Charophytologists (GEC), València, Spanien.

Blindow, I., Henningsen, L., **Herbst, A.**, Schubert, H., 2017. Incrustation of charophytes: differences among environmental parameters and species, 21th Meeting of the Group of European Charophytologists (GEC), València, Spanien.

Herbst, A., 2017. Kalziumkarbonat Ausfällung von Characeen – Saisonalität und Gewässerparameter, Deutsche Characeen Tagung, Lebus, Deutschland.

Herbst, A., Kohlberg, G., Niedzwiedz, G., 2016. Non-invasive longtime observation of the filtration activity of the freshwater mussel *Diplodon chilensis* (Gray, 1828) with time lapse photography, 2nd European Conference on Scientific Diving, Kristineberg, Schweden.

IT-skills

very good	R, LaTeX and Microsoft Office
basic	QGIS, SPSS

Stays abroad

03/2015 and 03/2016	**Chile**, *cooperation with the University of Valdivia*, project: AquaCare, time lapse photography for in situ observation of a Chilean bivalve using scientific diving.
08/2008 – 06/2009	**Australia und New Zealand**, intention: improvement of the language, exploring other cultures and societies.

Languages

English	fluent
French	advanced basics
Sanish	basics